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Application of cavalieri principle in patients with chronic subdural hematoma using CT scanning images

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Abstract

Introduction: Stereology provides techniques to evaluate three dimensional structures using their two dimensional images. The Cavalieri principle is one of these methods which allow getting volume data both using microscopic and macroscopic structures' images. The presented study aimed to describe the use of the unbiased Cavalieri principle, to asses Chronic subdural hematoma (CSDH) volume to total brain volume fraction and compare it with the patients' clinical features.

Methods: This study is a retrospective analysis of consecutive 101 patients with intracranial hematoma. The study included a final cohort of 28 patients who had only unilateral CSDH. Relationship between the percent of CSDH volume and patients' demographic and clinical features were evaluated with one-way variance analysis and/or spearman rank correlation analysis.

Results: There were 101 possible admissions for review, 28 patients meeting study criteria for review. Seventy three patients were excluded from the study. The average GCS was 13.9 ± 2.1 (range 5-15). While the mean volume of CSDH was 80.2 ± 43.8 cm³, the percent of CSDH volume was 6.3 ± 3.4 cm³. The correlation between midline shift and percent of CSDH volume was significant. There was a significant positive correlation between the percent of CSDH volume and age.

Conclusion: The present study demonstrated that percent of CSDH volume of patients was re-

lated to only age and midline shift. To the best of our knowledge, stereologic technique for calculating the CSDH volume has been reported for the first time. We believe that this study will light the way for larger study series on stereologic working about CSDH.

Keywords: Stereology, subdural hematoma, volume.

Introduction

Chronic subdural hematoma (CSDH) represents one of the most frequent intracranial hematomas, and is well known as a curable disease.^{1, 2} CSDH has a high incidence in the elderly population of many countries. In patients with symptomatic CSDH and focal neurologic deficits, surgical treatment should be considered. Asymptomatic patients or patients with only mild headaches may be followed up with conservative treatment and serial neuroimaging by computed tomography (CT). Despite early diagnosis and adequate resources, mortality and morbidity in subdural hematomas remain considerable. Bedside determination of the volume of CSDH may be useful in the patient management and prognostication in the emergency department. ABC/2 or XYZ/2 method is a simple estimation method of intracerebral hematoma volume. In this method, "A" indicates depth of hematoma that was determined by multiplying the number of slices on which hematoma was visible, "B" indicates maximum length of hematoma and "C" indicates maximum width of hematoma.³⁻⁶ Gebel et al. reported that ABC/2 technique is a simple, accurate bedside method for the measurement of subdural hematoma volume.⁷

Stereology provides techniques to evaluate three dimensional structures using their two dimensional images.⁸ The Cavalieri principle is one of these methods which allow getting volume data both using microscopic and macroscopic structures' images.9, 10 For using the Cavalieri principle, the interested object is cut in parallel plane sections from end to end, beginning at a random chosen starting point. This requirement of the Cavalieri principle meets the standard magnetic resonance imaging (MRI) (or CT) scans.¹¹⁻¹³ The surface area of the section is estimated using point-counting grid (PCG). The PCG is a set of points at constant distances printed on transparent sheet. The density of points must be sufficient to provide proper sampling. Reliability of point density of the PCG can be evaluated by calculating the coefficient of error (CE). The formula reported by Gundersen and Jensen can be used for calculating the CE of volume estimations.¹⁴ In Cavalieri principle the PCG is superimposed on sections in a random manner and the number of points that hits the region of interest is counted.^{11, 15-17} The numbers of points hitting the region of interest are multiplied by the representing area of each point to obtain the sectional cut surface area. The surface area of each section estimated and multiplied by the section thickness (or mean section thickness). The sum of all sections volume is the volume of interested

Figure 1. A combined point counting grid (CPCG) with 1/4 area fraction. Coarse points (encircled) represents a large area which is 4 times bigger than the area represented by fine points (not encircled point)

object. This technique needs the reduction/magnification factor to be known. This is the simple and reliable volume estimation method called Cavalieri principle.^{12, 17, 18} When the issue is estimating a component's volume fraction in the total object volume, combined point-counting grid (CPCG) is used instead of standard PCG. A CPCG has two sets of points each of different densities. Because the component's size is smaller than the whole object, less dense PCG is suitable for the reference structure but not for the component. Each point sets density must be adjusted according to the size of component's cut surface area. This gives appropriate CE for each component. The CPCG has two sets of point of different densities. One set represented with crosses (fine points) and the other with encircled crosses (coarse points). It should be considered that encircled points are used as both fine and coarse points. As the definition point is defined with zero dimensions, it must be showed with two intersected lines or crosses (Figure 1). For a CPCG the relation between represented area by fine and coarse points must be described. We used the CPCG illustrated at Figure 1. It has four fine points (crosses and encircled crosses) per coarse point. This grid could be described as a CPCG with 1/4 area fraction. We used the definition "volume fraction" to express the proportion of a component within the whole structure, as described by Kalkan et al. and Reed et al.^{19, 20} The presented study aimed to describe the use of the unbiased Cavalieri principle, to asses CSDH volume to total brain volume fraction and compare it with the patients' clinical features.

Material and method

Setting and Selection of Participants

This study is a retrospective analysis of consecutive 101 patients with intracranial hematoma who had been admitted to the Duzce University School of Medicine Hospital and Recep Tayyip Erdogan University School of Medicine Hospital. Exclusion criteria were the presence of (1) associated subarachnoid hemorrhage, (2) associated epidural hematoma, (3) associated acute subdural hematoma, (4) associated bilateral CSDH, or (5) increased level of International Normalized Ratio (INR). The study included a final cohort of 28 patients who had only unilateral CSDH. Medical records were independently reviewed for patient age, gender, time of admission, time of discharge or death, cause of subdural hematoma (traumatic or spontaneous), past medical history (hypertension, heart disease, stroke, diabetes mellitus, and other) and use of antiplatelet therapy. Systolic arterial pressure, diastolic arterial pressure, Mean arterial pressure (MAP), INR, type of treatment (conservative or surgical intervention), length of hospital stay (LHS) and presence of the midline shift were recorded. Neurological function on admission was measured by Glasgow Coma Scale (GCS), which was recorded on the life squad of emergency department records.

Stereological Estimations

We placed the CPCG over the section series, counted the number of coarse points that hits all of the brain area including the hematoma area, and counted the number of fine points hitting only the hematoma (Figure 2). Because of the proportion of the fine to the coarse points is 1/4 in the CPCG, the volume fraction of hematoma within all of the brain, estimated with this equation:

$$V_V(H,B) = \frac{\sum P_H}{4 \times \sum P_B}$$



Figure 2. The common point counting grid (CPCG) superimposed over a CT scan. While only coarse points (encircled) hitting the all of intracranial structure were counted, coarse and fine points which hitting the hematoma were counted

where V_v (H, B) is the volume fraction of hematoma to brain volume, $\sum P_H$ is total number of points that hit the hematoma area, $\sum P_B$ is the total number of points that hit all brain including hematoma. It must be considered that the 4 in the denominator is because of the area fraction of the CPCG that we used. In this approach the only required are the number of points hitting the hematoma and the number of points hitting all of intracranial structures including hematoma. This approach is not affected by the reduction/magnification rate of the images.¹⁹ The CE and volume fraction estimations were performed with a spreadsheet using Microsoft Excel 2007, which was previously prepared for this purpose.

Statistical Analysis

The data were analyzed using "Statistical Package for Social Sciences 15.0 for Windows" (SPSS-15) software. Kolmogorov-Smirnov test was used for checking the accordance of percent of CSDH volume to normal distribution. Data were expressed as frequencies, percents, and means (with standard deviation). Relationship between the percent of CSDH volume and patients' de-

Table 1. Demographic Features of Patients

Patients Cha	aracteristics	N / Mean	% / SD
Age (year)		70.8	15.0
LHS (day)		9.4	6.3
GCS		13.9	2.1
	Systolic TA	137.5	15.9
TA (mmHg)	Diastolic TA	87.5	11.6
	MAP	104.2	11.5
Valuma	Hematoma	80.2	43.8
$\sqrt{01}$ u m e	Whole Brain	1266.0	139.7
(cm ²)	Percentage	6.3	3.4
Condor	Male	19	67.9
Gender	Female	9	32.1
Cause of	Trauma	9	32.1
Hematoma	Spontaneous	19	67.9
Shift	Yes	22	78.6
Shift	No	6	21.4
Traatmont	Surgery	25	89.3
Treatment	Conservative	3	10.7
Quitaama	Discharge	27	96.4
Outcome	Died	1	3.6

LHS: Length of Hospital Stay, TA: Tension Arterial, MAP: Mean Arterial Pressure, GCS: Glasgow Coma Scale mographic and clinical features were evaluated with one-way variance analysis and/or spearman rank correlation analysis. Chi-square and student t test were used for comparisons of categorical and continuous variables, respectively.

Results

There were 101 possible admissions for review, 28 patients meeting study criteria for review. Seventy three patients were excluded from the study; 28 because of the presence of associated subarachnoid hemorrhage, 23 because of the presence of associated epidural hematoma, 16 because of the presence of associated acute subdural hematoma, 4 because of bilateral CSDH, and 2 because of the increased level of INR. The average age of these patients was 70.8±15.0 years, with a range of 1 month to 18 years; 19 were men and 9 were women. Twenty five patients (89.3%) had undergone surgical therapy. The overall mean LHS was 9.4±6.3 days, extending from 2 day to 30 days. The average GCS was 13.9±2.1 (range 5-15). While the mean volume of CSDH was 80.2±43.8 cm³, the percent of CSDH volume was 6.3 ± 3.4 cm³ (Table 1). Relationship between patients' clinical features and percent of CSDH volume was shown in Table 2. There were no statistical significant relationship between past medical history, cause of hematoma, treatment, gender and percent of CSDH volume (p>0.05). Only the correlation between midline shift and percent of CSDH volume was significant (p=0.003).

There was a significant positive correlation between the percent of CSDH volume and age (r=0.427 and p=0.023) (Figure 3). There were negative correlations between GCS and LHS with the percent of CSDH volume, but these were not statistically significant (r=-0.205 and p=0.30, r=-0.274 and p=0.16, respectively).

Discussion

Design based-stereology has become the stateof-the-art methodology to get knowledge about the brain in recent years ¹⁷. Various techniques can be used for volume estimations including planimetry,



Figure 3. Positive correlation between the percent of CSDH volume and age

Clinical Features		N	Mean Percentage of	SD	n	
		1	CSDH Volume (cm ³)	(cm ³)	Р	
	None	7	6.36	3.17		
	Hypertension	8	6.04	1.35		
Past Medical History	Heart Diseases	1	8.99		NS	
	Diabetes Mellitus	2	4.09	1.88		
	Other	10	6.70	3.74		
	Trauma	9	4.72	2.72	NS	
Cause of Hematoma	Spontaneous	19	7.08	3.48		
Midling Shift	Yes	22	7.25	3.19	0.002	
	No	6	2.91	1.32	0.003	
Treatment	Surgery	25	6.50	3.37	NC	
Treatment	Conservative	3	4.81	3.86	IND	
C 1	Male	19	6.94	3.29	NC	
Gender	Female	9	5.01	3.41	112	

Table 2. Relationship between Patients' Clinical Features and Percentage of CSDH Volume

SD: Standard Deviation, NS: Not Significance

image analysis programs or radiological imaging system software.^{1, 15, 16, 21, 22} But in printed images the options is limited.^{11, 23} We used the Cavalieri principle, an unbiased stereological technique that provides volume estimation. The technique can be applied on printed or monitor views of ultrasound images, standard CT or MRI scans .^{1, 21, 23, 24} Special trained staff, additional equipment or software is not needed.

There is some studies investigated relationship between intracranial hematoma volume and patients' clinical features.^{6, 25} In these studies, ABC/2 or computer-assisted volumetric analysis techniques had been used. Cavalieri principle had been used for epidural hematoma by Kalkan et al.¹⁹ This technique has not been used for calculation of CSDH volume up to date. Also, unlike other studies, patients' clinical features were compared with percent of CSDH volume in presented study. In terms of prognosis, the following parameters were found to be significant: age, time from injury to treatment, presence of pupillary abnormalities, GCS/motor score on admission, immediate coma or lucid interval, CT findings (hematoma volume, degree of midline shift, associated intradural lesion, compression of basal cisterns), post-operative intracranial pressure and the type of surgery.³

Schneck et al. reported that 63% of patients with subdural hematoma underwent surgical evacuation.²⁶ In the present study, we found that 86% of the patients were treated with surgery.

Midline shift was seen in 86% of the patients with CSDH.² In another study, midline shift was found in 61% of the patients with non-traumatic subdural hematoma.²⁶ In accordance with these reports, midline shift was detected in 78.6% of our patients. Ikeda et al. reported that the degree of midline shift in CSDH was not always correlated with hematoma volume.²⁷ Herein, mean percent of CSDH volume was higher in patients with midline shift.

Van den Brink et al. suggested that volume of the hematoma did not correlate with preoperative neurological condition.²⁸ Likewise, there was no statistical significant correlation between GCS on admission and percent of CSDH volume in our study. Schneck et al. reported that mean subdural hematoma volume was 97 mL on the left side and 82 mL on the right side.²⁶ Massaro et al. suggested in their series that GCS score and hematoma size were both important.²⁹ However, van Havenbergh et al. reported that CT findings such as hematoma volume, midline shift and residual subdural collections had no influence on the outcome. The only statistically significant factor for the outcome of patients with CSDH was the neurological condition at the time of treatment.³⁰ Ooba et al. reported that CSDHs in the extremely aged patients were characterized by severe degree of motor paralysis at admission, larger amount of drainage volume and low incidence of outcome improvement.³¹ This was consistent with our study. In our study, average age of patients was 70 years, and there was a significant positive correlation between the percent of CSDH volume and age.

There are some limitations in our study; the small sample size and retrospective design obliges us to be cautious in the generalization of the results. Relation between percent of CSDH volume and patients' outcome was not evaluated in the current study since there was a single death in the clinical follow up.

In conclusion, the present study demonstrated that percent of CSDH volume of patients was related to only age and midline shift. There were insignificant negative correlations between GCS and LHS with the percent of CSDH volume. To the best of our knowledge, stereologic technique for calculating the CSDH volume has been reported for the first time. We believe that this study will light the way for larger study series on stereologic working about CSDH.

References

- 1. Asghar M, Adhiyaman V, Greenway MW, Bhowmick BK, Bates A. Chronic subdural hematoma in the elderly-a north wales experience. J R Soc Med 2002;95(6):290-292.
- 2. Kim JH, Kang DS, Kim JH, Kong MH, Song KY. Chronic subdural hematoma treated by small or large craniotomy with membranectomy as the initial treatment. J Korean Neurosurg Soc 2011;50(2):103-108.
- 3. Servadei F. Prognostic factors in severely head injured adult patients with acute subdural haematoma's. Acta Neurochir (Wien). 1997;139(4):279-285.
- 4. Kwak R, Kadoya S, Suzuke T. Factors affecting the prognosis of thalamic hemorrhage. Stroke 1983;14:493– 500.

- 5. Kothari RU, Brott TG, Broderick JP, Barsan WG, Sauerbeck LR, Zuccarello M, et al. The ABCs of measuring intracranial hemorrhage volumes. Stroke 1996;27(8):1304-1305.
- 6. Broderick JP, Brott TG, Duldner JE, Tomsick T, Huster G. Volume of intracerebral hemorrhage: a powerful and easy-to-use predictor of 30-day mortality. Stroke 1993;24(7):987-993.
- Gebel JM, Sila CA, Sloan MA, Granger CB, Weisenberger JP, Green CL, et al. Comparison of the ABC/2 estimation technique to computer-assisted volumetric analysis of intraparenchymal and subdural hematomas complicating the GUSTO-1 trial. Stroke 1998;29(29):1799-1801.
- 8. Howard CV, Reed MG. Unbiased stereology. Threedimensional measurement in microscopy. 1998: Oxford: Bios. 55-56.
- 9. Sonmez OF, Odaci E, Bas O, Colakoglu S, Sahin B, Bilgic S, et al. A stereological study of MRI and the Cavalieri principle combined for diagnosis and monitoring of brain tumor volume. J Clin Neurosci 2010;17(12):1499-1502.
- Sarsilmaz M, Kaplan S, Songur A, Colakoglu S, Aslan H, Tunc AT, et al. Effects of postnatal formaldehyde exposure on pyramidal cell number, volume of cell layer in hippocampus and hemisphere in the rat: a stereological study. Brain Research 2007;11(1145):157-167.
- 11. Sahin B, Emirzeoglu M., Uzun A, Incesu L, Bek Y, Bilgic S, et al. Unbiased estimation of the liver volume by the Cavalieri principle using magnetic resonance images. Eur J Radiol 2003;47(2):164-170.
- 12. Roberts N, Puddephat MJ, McNulty V. The benefit of stereology for quantitative radiology. Br J Radiol 2000;73(871):679-697.
- 13. Roberts N, Cruz-Orive LM, Reid NM, Brodie DA, Bourne M, Edwards RH. Unbiased estimation of human body composition by the Cavalieri method using magnetic resonance imaging. J Microsc 1993;171(Pt 3):239-253.
- 14. Gundersen HJ, Jensen EB. The efficiency of systematic sampling in stereology and its prediction. J Microsc 1987;147(Pt 3):229-263.
- 15. Cruz-Orive LM. Stereology of single objects. J Microsc 1997;186:93-107.
- 16. Rosen GD, Harry JD. Brain volume estimation from serial section measurements: a comparison of methodologies. J Neurosci Methods 1990;35(2):115-124.

- 17. Schmitz C, Hof PR. Design-based stereology in neuroscience. Neuroscience 2005;130(4):813-831.
- 18. Mackay CE, Pakkenberg B, Roberts N. Comparison of compartment volumes estimated from MR images and physical sections of formalin fixed cerebral hemispheres. Acta Stereol 1999;18:149-159.
- 19. Kalkan E, Cander B, Gul M, Girisgin S, Karabagli H, Sahin B. Prediction of prognosis in patients with epidural hematoma by a new stereological method. Tohoku J Exp Med 2007;211(3):235-242.
- 20. Reed MG, Howard CV. Stereological estimation of covariance using linear dipole probes. J Microsc 1999;195(Pt 2):96-103.
- 21. Sahin B, Alper T, Kokcu A, Malatyalioglu E, Kosif R. Estimation of the amniotic fluid volume using the Cavalieri method on ultrasound images. Int J Gynaecol Obstet 2003;82(1):25-30.
- 22. Eriksen N, Rostrup E, Andersen K, Lauritzen MJ, Fabricius M, Larsen VA, et al. Application of stereological estimates in patients with severe head injuries using CT and MR scanning images. Br J Radiol 2010;83(988):307-317.
- 23. Bilgic S, Sahin B, Sonmez OF, Odaci E, Colakoglu S, Kaplan S, et al. A new approach for the estimation of intervertebral disc volume using the Cavalieri principle and computed tomography images. Clin Neurol Neurosurg 2005;107(4):282-288.
- 24. Mazonakis M, Karampekios S, Damilakis J, Voloudaki A, Gourtsoyiannis N. Stereological estimation of total intracranial volume on CT images. Eur Radiol 2004;14(7):1285-1290.
- 25. Sucu HK, Gokmen M, Gelal F. The value of XYZ/2 technique compared with computer-assisted volumetric analysis to estimate the volume of chronic subdural hematoma. Stroke 2005;36(5):998-1000.
- 26. Schneck MJ, Maheswaran M, Leurgans S. Predictors of outcomes after nontraumatic subdural hematoma. J Stroke Cerebrovasc Dis 2004;13(5):192-195.
- 27. Ikeda K, Kano A, Hayase H, Yamashima T, Ito H, Yamamoto S. Relationship between Symptoms of Chronic Subdural Hematoma and Hematoma Volume or Regional Cerebral Blood Flow. Neurol Med Chir (Tokyo) 1984;24:869-875.
- 28. van den Brink WA, Zwienenberg M, Zandee SM, van der Meer L, Maas AI, Avezaat CJ. The prognostic importance of the volume of traumatic epidural and subdural haematomas revisited. Acta Neurochir (Wien). 1999;141(5):509-514.

- 29. Massaro F, Lanotte M, Faccani G, Triolo C. One hundred and twenty-seven cases of acute subdural haematoma operated on: Correlation between CT scan findings and outcome. Acta Neurochir (Wien) 1996;138:185-191 (suppl).
- 30. van Havenbergh T, van Calenbergh F, Goffin J, Plets C. Outcome of chronic subdural haematoma: analysis of prognostic factors. Br J Neurosurg 1996;10(1):35-39.
- 31. Ooba S, Shiomi N, Shigemori M. Clinical features and surgical results of chronic subdural hematoma in the extremely aged patients. No Shinkei Geka 2006;34(3):273-278.

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Comparison of Shore D microhardness obtained by fiberglass splints Splint-It in two spatial arrangements

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Abstract

Aim: The aim of this study was to compare the Shore D microhardness of two spatial arrangements of one type of fiberglass splint in combination with different warp material. Methods: The study material consisted of six types of samples that were a combination of two splints Split-It (Linke and check arrangement). As a warp material were used composite fillings: LCR, Flow-It, Dyract.

Shore instrument was used in the study and it was equipped with a head D. The samples were pressed with the load of 50N. Each sample was tested five times. The measurements were carried out at the top and bottom of the sample. The results were presented using the arithmetic average.

Results: The highest average value of Shore D microhardness (71.7 ShD) received a combination of composite material LCR and reinforcement Splint-It Linke with a parallel set of fibers. The lowest average value (41.4 ShD) revealed the sample constructed of splint Splint-It in the reticular form in combination with Dyract matrix.

Conclusions: 1. The height of Shore D microhardness parameter provides information about homogeneity of the composite material. 2. From the results it can be assumed that considerable complexity of the reinforcement fibers has a negative effect on the value of Shore D microhardness. 3. High initial density of the matrix material is a helpful factor in obtaining high values of Shore D microhardness.

Keywords: Teeth stabilization, Shore D microhardness, composite materials.

Introduction

A significant role of periodontium is the role involving the direct depreciation against the forces directed to the tooth during mastication. With the pressure of mild external force there is observed periodontal tooth mobility. It depends on different widths of the alveolus. This initial tooth mobility is not only the result of different width of periodontium, but also a function of its marginal fibers whih tighten at the pressure side. In the middle of the root, where periodontium is the narrowest -fiber tension is minimal. It is called periodontium physiological mobility by Rateitschak. When we press with a greater force there is also observed physiological mobility, but it shows the flexibility of the alveolar processus. It is mobility of the periodontium by Rateitschak [1,2]. As a result of local or systemic factors pathological mobility occurs. According to Entin we can specify the following degrees of tooth mobility:

I⁰ - visible and palpable mobility in the labial - lingual direction,

II⁰ - visible and palpable mobility in the labial - lingual direction and mesial – distal,

 III^0 - mobility in the vertical direction, along the long axis of the tooth [3].

Mobile teeth are quite common symptom among patients which are coming to dental office. The factors causing this state are mainly inflammatory processes in the marginal and periapical periodontium, but also we have:

- mechanical injuries,
- traumatic occlusion,
- orthodontic treatment.

One of the methods of conservative treatment is immobilization of mobile teeth. Tooth splinting is often a prelude to the next stage of treatment. It helps the weakened function of periodontium after periodontal microsurgery, by this it enables regeneration and prevent for further local damage [1]. The object of the study were the splints, which can be used for an extended period of time. Therefore, they must be those with very good mechanical properties as well as providing a satisfactory cosmetic result. One method of stabilization is the splint that uses the technique of enamel etching and adhesives. Stabilization splint consists of two elements: the reinforcement and matrix. Reinforcement are:

- ready-made preparations, such as multifilamentary tape made from fiberglass or fiberglass-reinforced chemically connecting with the various types of adhesive materials,

- metals (retainers),
- artificial materials.

They differ among themselves with the amount of fibers placed in a bunch and their cross-laying. Warp materials mostly are composite or compomers materials [4]. The stabilizing splints are sometimes fracturing, which result in detachment or rupture from the teeth. To limit the damage of the splint strength tests were carried out, because without knowledge of the mechanical parameters of materials it is impossible to connect them accordingly in order to obtain the minimum number of complications.

N f	Material					
sample	Reinforcement	Wrap material				
1	Splint-it (reticular arangement)	LCR				
2	Splint-it Linke	LCR				
3	Splint-it (reticular arangement)	Dyract				
4	Splint-it Linke	Dyract				
5 Splint-it (reticular arangement)		Flow – It				
6	Splint-it Linke	Flow – It				

Table 2. R	esults of Shore	e D microhardne	ess

Aim

The aim of this study was to compare the Shore D microhardness of two spatial arrangements of one type of fiberglass splint in combination with different warp.

Materials and methods

The study material consisted of six types of samples that are a combination of two reinforcements (Table 1.):

- Split-It in the form of check a width of 2 mm (Splint-It reticular alignment)
- Splint-It cord with parallel alignment with a 1 mm width(Splint-It Linke)

As a warp material composite fillings like LCR, Flow-It, Dyract were used. System Splint-It for teeth splinting offers three types of fibers: 1 mm cord, 2 mm reticular fiber alignment and 3 mm fibers with parallel arrangement. Reinforcement Splint-It is ready for use - impregnated with resin at the production stage fiberglass [5]. Samples were prepared in the form of beams with dimensions of 25x4x1.7 mm. These were taken on a specially made plexiglass module according to the manufacturer's instructions, in a manner analogous to the clinical application conditions. Samples were cured with Heraeus - Kulzer lamp. Each sample was trated for 60 seconds (Figure. 1). Microhardness testing was carried out with use of Shore hardness tester (Figure. 2) Microhardness we study using the rod ended with cone. Measuring range depends on the hardness of the material. There are two types of measuring heads A and D. A range is used for materials with a hardness from 10 to 90 ° Shore A, while the range of D is used in materials with a hardness from 30 to 90 ° ShD.

No.of	Material	Microhardnes, ShD			
sampla	Reinforcement	Wrap	Top of	Bottom of	Average
sampic	Remoreement	material	sample	sample	1 wer age
1	Splint-it (reticular arrangment)	LCR	56.3	54.7	55.5
2	Splint-it Linke	LCR	77.6	65.8	71.7
3	Splint-it (reticular arrangment)	Dyract	40.8	42.0	41.4
4	Splint-it Linke	Dyract	57.8	56.0	50.9
5	Splint-it (reticular arrangment)	Flow – It	42.4	46.6	44.5
6	Splint-it Linke	Flow - It	51.4	49.6	50.5

With these two types of heads are also connected various loads. Durometer A scale is loaded with a force of 12.5 N. The head D requires a 50N load. This device is equipped with a dial gauge. During the tests we read the measured values from the above-mentioned sensor. The sample is placed at a distance of 5-13 mm from the pin, which is set perpendicular to the surface being tested [6]. The measurements were performed using the head D with load 50N. In this way, all the samples were tested. The surface of each was tested five times. The arithmetic average was taken for these measurements.



Figure 1. Photography of plexiglass module



Figure 2. Shore hardness test device

Results

The highest average value of microhardness Shore D (71.7 ShD) received connection of LCR matrix and the reinforcement Splint-It Linke with a parallel set of fibers. The lowest average value (41.4 ShD) showed sample of Splint-It reticular type with warp Dyract.. The highest value of microhardness Shore D at the bottom of the sample (65.8 ShD) achieved a connection of Splint-It Linke with a parallel set of fibers and warp LCR. The lowest value of microhardness Shore D at the bottom of the sample (42 ShD) obtained a sample made from Splint-It with reticular arrangement connected with Dyract matrix. At the top of sample the highest value of Shore D microhardness received sample built from Splint-It Linke and matrix material LCR. The lowest value (40.8ShD) in the upper part of the sample had connection of Splint-It (reticular type) reinforcement with Dyract warp. When Flow-It composite was used as a warp, Shore D microhardness value was similar to that obtained in samples using matrix Dyract (Tab. 2).

Conclusions

1. The height of Shore D microhardness parameter provides information about homogeneity of the composite material.

2. From the results it can be assumed that considerable complexity of the reinforcement fibers has a negative effect on the value of Shore D microhardness.

3. High initial density of the matrix material is a helpful factor in obtaining high values of Shore D microhardness.

References

- 1. Górska R.: Etiopatogeneza. in: Praktyczna periodontologia kliniczna. Red. Naukowa Jańczuk Z., Kwintesencja 2004, 2, 23-35.[in Polish]
- 2. Górska R.: Choroby przyzębia. Akademia Medyczna. Warszawa, 2001.[in Polish]
- 3. Knychalska –Karwan Z.: Stomatologia geriatryczna. Collegium Medicum UJ. 1995.[in Polish]

- 4. Jańczuk Z., Borysewicz G.: "Unieruchomianie rozchwianych zębów metodą Splint-Lock i Fiber-Splint" Med. Tour Press International. 1996.[in Polish]
- 5. http://jenerix.com.pl/?s=5&prodID=19&infoID
- 6. Konowalski K.: Ćwiczenia Laboratoryjne z wytrzymałości materiałów. Politechnika Szczecińska Katedra Mechaniki i Podstaw Konstrukcji Maszyn, Szczecin 2005.[in Polish]

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Cyclooxygenase-2 promoter polymorphism is related to genetic predisposition to non-alcoholic fatty liver disease

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Abstract

Introduction

Objective: To investigate whether Cyclooxygenase-2 promoter single nucleotide polymorphism (SNP) is related to genetic predisposition to nonalcoholic fatty liver disease (NAFLD).

Methods: Polymerase chain reaction - restriction fragment length polymorphism was employed to measure the SNP in COX-2 promoter (-765G>C and -1195A>G) in NAFLD patients and healthy subjects. Quantitative data were compared with t test following test of homogeneity of variance. Comparisons of gender, genotype and allele frequency were done with chi square test.

Results: The allele frequency of -1195G>A and -765 G>C was 0.48 and 0.02, respectively, in healthy subjects and 0.54 and 0.05, respectively, in NAFLD patients. Multivariable Logistic regression analysis showed the risk for NAFLD in -765GC carriers (OR=2.35, 95%CI: 1.17~3.65) was higher than that in -765GG carriers and the risk for NAFLD in -1195AA carriers was higher than that in -1195GG carriers (OR=1.13, 95%CI: $1.01 \sim 2.46$). When compared with subjects with haplotype G_{-1195} - G_{-765} , subjects with haplotype A_{-1195} or A^{-1195} - C_{-765} , A_{-119} 5- G_{-765} had significantly increased risk for NAFLD (OR: 1.42, 95% CI: 1.11~1.63, P<0.05; OR: 4.24, 95% CI: 1.72~14.22, P<0.01), and the risk for NAFLD in subjects with haplotype A-1195-C-765 was higher than that in subjects with haplotype A₋₁₁₉₅-G₋₇₆₅,G₋₁₁₉₅-C₋₇₆₅. These findings suggest there was interaction between -1195A and -765C.

Conclusion: Our findings demonstrate the SNP in COX-2 promoter (-1195G>A and -765G>C) is related to the occurrence of NAFLD and an important factor determine the genetic predisposition to NAFLD.

Keywords: Fatty liver, Cyclooxygenase-2, Single nucleotide polymorphism.

The pathogenesis of nonalcoholic fatty liver disease (NAFLD) is complex and cyclooxygenase-2 (COX-2) has been found to play an important role in the development of fatty liver disease. The single nucleotide polymorphism (SNP) of COX-2 promoter may alter the binding ability of transcription factor leading to the change in transcription activity of this promoter. In the studies on cancers, the polymorphism of -1195G>A has been found to form c-myb binding site and may lead to increase in the transcription activity of COX-2 promoter in Hela cells. Moreover, subjects with -1195A allele or -765C allele are found to possess increased risk for cancers (1-2). However, whether polymorphism of -1195G>A and -765G>C may induce the alteration in transcription activity of COX-2 promoter in cells of NAFLD patients and the correlation between the polymorphism of both and NAFLD are rarely reported.

Materials and methods

Main reagents and instrument

Tris (hydroxymethyl) aminomethane (Tris base), Taq DNA polymerase, DNTP, buffer, agarose, 2000-bp DNA (Promega, USA), *Pvu* II, Rsa II (Biolabs, USA), GeneArip2400 thermocycler (Perkin-Elmer, USA), WP ultraviolet projection instrument (Yongjia Shangtang Teaching Instrument Factory), DY-B1 shaker (Jiangsu Xinghua Analysis Instrument Factory), DYCZ-28A electrophoresis tank (Beijing Liuyi Instrument Factory), microreader (450 nm; Sigma, USA) and UVP gel image analysis system (GDS-8000 UVP, USA) were used in the present study.

Patients

NAFLD inpatients and outpatients were recruited from the Second Affiliated Hospital of Xi'an

Jiaotong University from December 2006 to April 2007. NAFLD was diagnosed by findings in clinical and imaging examinations according to the criteria for the diagnosis of fatty liver disease and non-alcoholic liver disease developed by the Hepatology Branch of Chinese Medical Association (3). In addition, the healthy subjects for routine physical examination were also recruited. Both NAFLD patients and healthy subjects were Han Chinese living in Shangxi Province and had no kinship. Hepatitis B induced hepatic cirrhosis, alcoholic cirrhosis, diabetes and other metabolic diseases were excluded from NAFLD patients. The routine blood test and biochemical examinations revealed normal. Ultrasonography was done with LOGIQ500Pro Color Doppler Ultrasound to diagnose fatty liver disease. Informed consent was obtained from each subject before study.

Sample collection

Patients were fasted for 12 h and then the height and body weight were measured to calculate the body mass index (BMI) (BMI=body weight [kg] / height 2 [m²]). EDTA anti-coagulated venous blood (2 ml) was collected and stored at -20°C. Then, the plasma and blood cells were separated for the detection of COX-2 level and COX-2 genotype. In addition, another 5 ml of venous blood were also collected for used.

Primer design and procedures

The primers were as follows: -765G>C: 5'-ATTCTGGCCATCGCCGCTTC-3' (forward), 5'-CTCCTTGTTTCTTGGAAA-GAGAGG-3' (reverse); 1195A>G: 5'-TATGCTGTCATTTTCCTGTA -3' (forward), 5'- GTTTTGGAACATAGTTGGAT- 3' (reverse). These primers were synthesized in Shanghai Sangon Biotech Co., Ltd. The primers were prepared at -20°C and stored at -20°C. Polymerase chain reaction-based Restriction fragment length polymorphism (PCR-RFLP) was employed detect the genotype of -765G>C and -1195A>G in COX-2 promoter. Genotyping was done in a blind manner to assure the reliability of genotype. In addition, 10% of samples were randomly selected for genotyping again and results showed consistence with results in first genotyping.

Random selection of samples for sequencing for confirmation

Sequencing was performed with ABI PRISM 3730 sequencer (ABI, USA) and BigDye terminator v3.1 (Applied Biosystems, USA).

Statistical analysis

Haplo.score was employed to calculate the haplotype frequency of -765G>C and -1195A>G. LDA was used to calculate the linkage disequilibrium coefficient D and D'. Hardy-Weinberg equilibrium test was done to confirm the group representation. D represents the difference between general haplotype frequency and detected haplotype frequency; D' represents the standardized D (range: 0~1). D'=0: absence of linkage; D'=1: complete linkage; D'<1: linkage disequilibrium. Non-conditional logistic regression model was employed to calculate the odds ratio (OR) and 95% confidence interval (CI) to present the relative risk for NAFLD in patients with specific genotype. Haplo.glm was used to calculate the OR and 95% CI to present the relative risk for NAFLD in patients with specific haplotype. Statistic Analysis system 6.12 was employed for above analysis. Genotype was analyzed with direct gene counting method. Quantitative data were expressed as $\overline{x} \pm s$ and t test was done following test of homogeneity of variance. Gender, genotype and allele frequency were compared between groups with chi square test. A value of P<0.05 was considered statistically significant. SPSS version 12.0 was used for statistical analysis.

Results

Characteristics of subjects

A total of 200 NAFLD patients were recruited. There were 107 males and 93 females with a mean age of 54.4 ± 11.6 years (range: $21\sim74$ years). In the control group, 206 healthy subjects were enrolled. There were 106 males and 100 females with a mean age of 56.4 ± 12.3 years (range: $18\sim69$ years). The characteristics of NAFLD patients and healthy subjects are shown in Table 1. No marked differences were found in the gender and age between two groups (P>0.05). The BMI of NAFLD patients was higher than that of healthy subjects (t=2.914, P<0.05).

Genotyping with PCR-RFLP and Sequencing

Following enzyme cutting, COX-2 can be divided into 2 genotypes. The allele frequency of -765G>C and -1195A>G in COX-2 promoter was 0.48 and 0.02 in the healthy subjects and 0.54 and 0.05 in the NAFLD patients. For -765 G>C, the -765CC was the normal genotype and enzyme cutting occurred between C and C. When there was no -765G \rightarrow C mutation, the size of two fragments was 188 bp and 118 bp, respectively after enzyme cutting; when there was $-765G \rightarrow C$ mutation, both DNAs had mutations and could not separated, and the size of one fragment was 306 bp (CC) following enzyme cutting. In the present study, no homozygote was found. Following enzyme cutting, heterozygote fragments with 306 bp, 188 bp and 118 bp (CG) were identified, which suggested one DNA had mutation. Following enzyme cutting of -1195 A>G, the -1195AA was 27b bp in size; -1195AG was 230 bp and 276 bp in size and -1195GG was 230 bp in size. Forward sequencing showed consistent results with findings in PCR. Except for mutation sites, the remaining findings were in accordance with the results in Genebank.

-765G>C genotype in COX-2 promoter, allele frequency and risk for NAFLD

The -765CG frequency in NAFLD patients was markedly higher than that in healthy subjects (P<0.05). The -765CC frequency in NAFLD patients (15%) was markedly higher than that in healthy subjects (4.85%) (P<0.05). (Table 2). Multivariable logistic regression analysis showed the risk for NAFLD in -765CG carriers was markedly *Table 1. Gender, age and BMI in both groups*

increased when compared with -765GG carriers (OR=2.26, 95%CI: 1.17~3.65; P<0.05).

Genotyping with PCR-RFLP and Sequencing

Following enzyme cutting, COX-2 can be divided into 2 genotypes. The allele frequency of -765G>C and -1195A>G in COX-2 promoter was 0.48 and 0.02 in the healthy subjects and 0.54 and 0.05 in the NAFLD patients. For -765 G>C, the -765CC was the normal genotype and enzyme cutting occurred between C and C. When there was no -765G \rightarrow C mutation, the size of two fragments was 188 bp and 118 bp, respectively after enzyme cutting; when there was $-765G \rightarrow C$ mutation, both DNAs had mutations and could not separated, and the size of one fragment was 306 bp (CC) following enzyme cutting. In the present study, no homozygote was found. Following enzyme cutting, heterozygote fragments with 306 bp, 188 bp and 118 bp (CG) were identified, which suggested one DNA had mutation. Following enzyme cutting of -1195 A>G, the -1195AA was 27b bp in size; -1195AG was 230 bp and 276 bp in size and -1195GG was 230 bp in size. Forward sequencing showed consistent results with findings in PCR. Except for mutation sites, the remaining findings were in accordance with the results in Genebank.

-765G>C genotype in COX-2 promoter, allele frequency and risk for NAFLD

The -765CG frequency in NAFLD patients was markedly higher than that in healthy subjects (P<0.05). The -765CC frequency in NAFLD patients (15%) was markedly higher than that in he-

Group	n	Gender (n, %)		Age (years, n, %)			BMI (kg/m ²)
		Male	Female	≤40	40~60	>60	
Healthy subjects	206	106(± 100(4	51.5) 48.5)	49(23.8)	107(51.9)	50(24.3)	23.22±1.46
NAFLD patients	200	107(53.5)	93(46.5)	56(28)	99(49.5)	45(22.5)	26.06±2.42 ª

ap=0.026 vs control group (healthy subjects)

Table 2. -765G>C genotype in COX-2 promoter in two groups (n, %)

Group	n	GG%	CG%	С%	G%
Healthy subjects	206	97.09	2.91	4.85	95.15
NAFLD patients	200	89.00	11.00ª	15.00 ^b	85.00
					1 1

Note: NAFLD: nonalcoholic fatty liver disease; non-conditional logistic regression model was used for calculation following adjustment of gender and age; ${}^{a}P < 0.05$ vs healthy subjects; ${}^{b}P < 0.05$ vs healthy subject

althy subjects (4.85%) (P<0.05). (Table 2). Multivariable logistic regression analysis showed the risk for NAFLD in -765CG carriers was markedly increased when compared with -765GG carriers (OR=2.26, 95%CI: $1.17\sim3.65;$ P<0.05).

-1195G>A genotype in COX-2 promoter, allele frequency and risk for NAFLD

The genotype frequencies in two groups are shown in Table 3. There was no significant difference in the -1195GG and -1195GA between two groups (P>0.05, D=1). When compared with -1195GG carriers, -1195AA carriers had increased risk for NAFLD (OR=1.13, 95% CI: $1.01\sim2.46$; P<0.05).

Correlation between different haplotypes and risk for NAFLD

The haplotype significantly influences the phenotype, and thus Haplostats was employed to calculate the haplotype frequency of both genotypes (Table 4) and the risk (95% CI) for NAFLD in patients with different haplotypes. Significant differences in the frequencies of A_{-1195} - C_{-765} , A_{-1195} - G_{-765} , G_{-1195} - G_{-765} and G_{-1195} - C_{-765} were found between NAFLD patients and healthy subjects (X2=33.67, P < 0.01). When compared with patients with haplotype G₋₁₁₉₅-G₋₇₆₅, the risk for NAFLD was markedly increased in patients with haplotype A_{-1195} - C_{-765} and $A_{-119-5}G_{-765}$ (OR, 1.42, 95%CI: 1.11~1.63, P<0.05; OR: 4.24, 95%CI: 1.72~14.22, P<0.01). Moreover, the risk for NAFLD in patients with haplotype A-1195-C-765 was significantly higher than that in those with haplotype A_{-1195} - G_{-765} and G_{-1195} - C_{-765} (P<0.05). This suggested that there was interaction between -1195A and -765C in the same haplotype.

Linkage disequilibrium analysis of -1195G>A and -765 G>C in COX-2 promoter

LDA was employed to calculate the linkage disequilibrium coefficient (D'). Results showed the D' value was 0.0439 ($X^2=0.0519$, P=0.845), meeting the Hardy–Weinberg principle.

Discussion

The mechanism underlying the inflammation in NAFLD is still poorly understood. COX-2 can act as an adipocytokine with multiple bioactivities and bridges the lipodystrophy and inflammation (4). Studies have shown that COX-2 has clinical important SNP, which may affect the normal physiology and is found to be related to the blood lipids, insulin resistance, C reaction protein (a marker of inflammation), etc. The COX-2 synthesis is regulated by the polymorphism and COX-2 of different amounts may affect the susceptibility to and severity of diseases. Genetic mutation (mainly SNP) has the risk for inducing alteration in gene functions which may influence its phenotype including gene related diseases. Our results showed there were two SNPs in the COX-2 promoter (-765G>C and -1195G>A) with frequency of >5%, which was related to the risk for NAFLD. The substitution of G with C at -765 of COX-2 promoter may result in deficiency in the enzyme site (Rsa I, Pvu II) and the nucleotide sequences can not be cut by enzymes, which produces GG genotype (no mutation) and CG (heterozygote with mutation). The substitution of G with A at -1195 of COX-2 promoter results in GG, GA and AA genotypes.

Table 3. -1195G>A genotype in COX-2 promoter in two groups (n, %)

Group	n	GG	GA	AA
Healthy subjects	206	58(28.16)	119(57.77)	29(14.08)
NAFLD patients	200	32(16.00)	92(46.00)	76(38.00) ^a

Note: NAFLD: nonalcoholic fatty liver disease; non-conditional logistic regression model was used for calculation following adjustment of gender and age; ${}^{a}P < 0.05$ vs healthy subjects;

Table 4. Different haplotypes of COX-2 promoter in NAFLD patients and healthy subjects (%)

Group	n	G ₋₁₁₉₅ -G ₋₇₆₅	A ₋₁₁₉₅ -G ₋₇₆₅	A ₋₁₁₉₅ -C ₋₇₆₅	G ₋₁₁₉₅ -C ₋₇₆₅
Healthy subjects	206	186	10	4	6
NAFLD patients	200	142	46ª	12 ^b	0°

Note: non-conditional logistic regression model was used for calculation following adjustment of gender and age; ${}^{a}P < 0.05$ vs healthy subjects; ${}^{b}P < 0.01$ vs healthy subjects; c: no calculation

For most SNP sites, any subject has the possibility to carry any genotype, but the genotype frequency may vary among populations. The genotype frequency of COX-2 promoter is also different among different races. In the present study, the frequency of -1195 GG, GA and AA in COX-2 promoter was 28.16%, 57.77% and 14.08%, respectively, in healthy subjects, which were similar to the results in the study of Zhang et al (5) in Han Chinese (22.5%, 53.4% and 24.1%, respectively). In Indians of North America, the frequency of -1195 GG, GA and AA was 12.6%, 45.0% and 42.4%, respectively (6). In the present study, the frequency of -765 GG, GC and CC was 97.09%, 2.91% and 0%, respectively, in healthy subjects, which was comparable to that in the study of Zhang et al (5) in Han Chinese (95.7%, 4.3% and 0%, respectively), but different from that in other races.

Our results showed the frequencies of -765 GC genotype and -1195 AA genotype in NAFLD patients were markedly higher than those in healthy subjects. The frequency of -765 GC genotype was 11.0% in NAFLD patients, but 2.91% in healthy subjects. Multivariable analysis showed the risk for NAFLD in -765GC carriers was 2.26 folds higher than that in -765GG carriers, and the risk for NAFLD in -1195 AA carriers was 1.13 times higher than that in -1195 GG carriers. NAFLD is as a result of interaction of multiple genes and multiple factors. In the study on genetic mutation of common diseases, haplotype was found to provide more information than SNP, thus, currently, increasing studies have conducted for the combination analysis of multiple candidate genes or several SNPs, and haplotype analysis has been regarded as an effective method(7). Studies have confirmed that there is high correlation among different SNPs and thus analysis of several SNPs may used to confirm the specific haplotype. The occurrence of some combinations of alleles in a population more often than would be expected from a random formation of haplotypes from alleles based on their frequencies refers to linkage disequilibrium (8-9). A pair of sites with interval of 10 kb might be incomplete linkage disequilibrium, but the linkage correlation of sites with interval of several bp in the same region of chromosome is weak. Similarly, the scope of regions with linkage disequilibrium in the genome may vary in different genes (10-12). Our results showed two genetic mutations of COX-2 promoter had linkage disequilibrium. There were marked differences in the four haplotypes $(G_{-1195}-G_{-765}, A_{-1195}-C_{-765}, A_{-1195}-G_{-765})$ between NAFLD patients and healthy subjects. When compared with subjects with haplotype G_{_1195}-G_{_76}5, the risk for NAFLD in patients with haplotype A-1195-C-765 and A_{-1195} - G_{-765} was markedly increased. The risk for NAFLD in subjects with haplotype A_{-1195} -C₋₇₆₅ was also higher than that in subjects with haplotype A $_{1195}\text{-}G_{\text{-}765}$ and $G_{\text{-}1195}\text{-}C_{\text{-}765}$. This suggests there is interaction between -1195A and -765C in the same haplotype. Haplotype analysis showed the risk for NAFLD in patients with both -1195 A and -765 C further increased, suggesting that both mutations in the same chromosome could exert synergistic effect.

Our results confirm the SNPs of COX-2 promoter in Han Chinese and -1195AA genotype and -765CG genotype are the risk genotypes of NAFLD. However, there might be possibility that small sample size and race in the present study bias our results. Thus, more studies with large sample size are required to confirm the relationship between SNPs of COX-2 promoter and NAFLD in Han Chinese.

References

- 1. Courivaud C, Bamoulid J and Tiberghien P, et al. G-765C COX-2 gene promoter polymorphism and risk of atherosclerosis after kidney transplantation. Transplantation 2009; 88:851-852
- 2. Pandey S, Mittal RD, Srivastava M, Srivastava K, Mittal B. Cyclooxygenase-2 gene polymorphisms and risk of cervical cancer in a North Indian population. Int J Gynecol Cancer. 2010; 20:625-630
- 3. Group of Fatty Liver Disease and Alcoholic Liver Disease of Hepatology Branch of Chinese Medical Association. Guideline for the diagnosis and treatment of nonalcoholic fatty liver disease. Chin J Hepatol 2006; 14:161-163
- 4. Cao MB, Dong L, Lu XL, Luo JY. An experimental study on the expression and pathogenic effects of cyclooxygenase-2 in nonalcoholic fatty liver disease. Zhonghua Gan Zang Bing Za Zhi. 2008; 4: 315-316

- 5. Zhang X, Miao X, Tan W, et al. Identification of functional genetic variants in cyclooxygenase-2 and their association with risk of esophageal cancer. Gastroenterology. 2005; 129: 565-576
- 6. Lowenfels AB, Maisonneuve P, Whitcomb DC, Lerch MM, DiMagno EP. Cigarette smoking as a risk factor for pancreatic cancer in patients with hereditary pancreatitis. JAMA 2001; 286: 169-170
- 7. Li AL, Song YX, Wang ZN, et al. Polymorphisms and a haplotype in heparanase gene associations with the progression and prognosis of gastric cancer in a northern Chinese population. 2012; PLoS One. 7:e30277
- 8. Nanji AA, Miao L, Thomas P, et al. Enhanced cyclooxygenase-2 gene expression in alcoholic liver disease in the rat. Gastroenterology 1997; 112:943-951
- Enomoto N, Ikejima K, Yamashina S, et al. Kupffer cell-derived prostaglandin E(2) is involved in alcohol-induced fat accumulation in rat liver. Am J Physiol Gastrointest Liver Physiol 2000; 279:G100-106
- 10. Gasparini G, Longo R, Sarmiento R, Morabito A. Inhibitors of cyclo-oxygenase 2: a new class of anticancer agents? Lancet Oncol. 2003; 4:605-615
- 11. Hla T, Bishop-Bailey D, Liu CH, et al. Cyclooxygenase-1 and -2 isoenzymes. Int J Biochem Cell Biol. 1999; 31(5):551-7
- Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, Simmons DL. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc Natl Acad Sci U S A. 2002; 99:13926-13931.

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Beneficial effects of preprocedural Trimetazidine on periprocedural myocardial damage

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Abstract

Objectives: In this study, we sought to determine the beneficial effects of preprocedural trime-tazidine on periprocedural myocardial damage.

Background: A common acceptance exists that the myocardial damage appeared after percutaneous coronary intervention (PCI), is related to adverse events in patients short and long term follow-up. Post-PCI serum creatine kinase and troponin levels are helpful to determine the size of myocardial damage. In order to decrease the damage, several agents had been tried. Trimetazidine is an anti-ishemic agent with metabolic effects. Drug regulates the energy metabolism of damaged myocardial cells which encountered hypoxia and ischemia.

Methods: Sixty consecutive PCI patients (71% men, 54±9 years) whom were on trimetazidine therapy were enrolled into the study. And patients (n:60, 68% men, 52±10 years) whom were not on trimetazidine was matched as the control group. All patients' baseline creatine kinase-MB (CKMB), troponin I (tnI) and high-sensitive C-reactive prote-in (hsCRP) samples were obtained. After the procedure, 6., 12. and 24. hour CKMB and tnI and only 24. hour hsCRP samples were also obtained and the highest values were recorded.

Results: Two groups were matched based on the basic demographic characteristics. One patient of the study group was excluded. General features and the mean values of baseline CK-MB, troponin I and hsCRP levels did not significantly differ between the two groups (p>0.05). Among the post-procedural mean values of CKMB and TnI levels, the values at the 24. hour were found to be the highest, and taken in to account for comparison with the pre-procedural values. The post-procedural rise in the mean value of CKMB (Δ CKMB) and mean value of hsCRP (Δ hsCRP) within 24 hours was significantly higher in group 2 compared to that in group 1 (p <0.0001) while the post-procedural rise in the mean value of troponin I (Δ troponin I) within 24 hours was insignificantly higher in group 2 compared to that in group 1 (p>0.05).

Conclusion: The present study clearly demonstrated the beneficial and protective effects of pre-procedural trimetazidine administration on myocardium in patients undergoing elective uncomplicated PCI. Future prospective randomized studies are still warranted to examine the effects of trimetazidine on adverse clinical outcomes and survival in patients undergoing PCI.

Key words: Percutaneous coronary intervention, Trimetazidine, Myocardial damage.

Introduction

Elevation of serum levels of total serum creatine kinase (CPK) and MB isoenzyme fraction (CKMB) have been relatively common after successful PCI¹ and is related to myocardial damage. Some studies have particularly demonstrated the close relation between even mildly elevated serum CK-MB levels and adverse long term prognosis in patients undergoing PCI². In the last few years, relatively more sensitive and more spesific markers of myocardial damage including troponin I and troponin T have come in to clinical practice. Troponin I was found to be the most sensitive marker to evaluate minor myocardial damage associated with interventional cardiac procedures³.

Free oxygen radicals (FOR) have major contribution to the emergence of PCI-related myocardial damage. The major initiators of FOR formation are acute ischemia and reperfusion of the PCIrelated myocardial territory. Elevated levels of FOR due to PCI are known to induce synthesis of some pro- inflammatory mediators including C-reactive protein (CRP), tumor necrozing factor alpha (TNF-alpha), interleukin -1 (IL-1). Therefore, these mediators are also closely associated with myocardial injury, and may mirror PCI-related myocardial damage.

The present study was devised to demonstrate the effects of pre-procedural trimetazidine, an anti-ischemic agent, on PCI-related myocardial damage as evaluated with serum levels of three important markers: CK-MB, Troponin I and CRP.

Methods

Sixty consecutive PCI patients with de novo native proximal or near-proximal coronary lesions (71% men, 54±9 years) whom were on trimetazidine therapy for stable angina pectoris were enrolled into the study. And patients (n:60, 68% men, 52 ± 10 years) whom were not on trimetazidine was matched as the control group. The presence of elevated serum baseline CKMB, troponin I and hs-CRP levels (above the upper limits), the presence of recent myocardial infarction(MI), recent history of any cardiac surgery (within the last 3 weeks) or the presence of significantly low left ventricular ejection fraction (LVEF) (LVEF<30%), the presence of significant left main coronary stenosis, total occlusions (TIMI grade 0), the presence of known hypersensitivity to TMZ, and the presence of hepatic or renal failure (TMZ metabolized in liver and excreted via urine) were accepted as exclusion criteria. All patients in both groups received clopidogrel with loading doses of 300 mg at least 2 hours before the procedure. Post-procedural antiplatelet regimen includes aspirin at 100 mg/day indefinitely and clopidogrel 75 mg/day for at least one month. Venous serum samples were drawn from patients in both groups before and after PCIs to measure the serum concentrations of creatin kinase-MB (CKMB), troponin I (tnI) and high- sensitive C-reactive protein (hsCRP). The pre-procedural venous samples were drawn just before clopidogrel loading. After the PCIs, venous samples to measure CKMB (at 6., 12., 24. hour), TnI (at 6., 12., 24. hour) and, hsCRP (at 24. hour) concentrations were also drawn from patients in both groups. Among the mean post-procedural values (6., 12. and 24. hour), the highest value was taken in to account in both groups for comparison with the pre-procedural values. The 2 groups were compared with each other in terms of post-procedural rise (the difference between post and preprocedural values) in the mean values of CK-MB, troponin I and hsCRP levels (Δ CKMB, Δ troponin I, Δ hsCRP). In our local laboratory, the normal upper limits of CKMB and Tn I are 6 mg/dL and 0.01 mg/dL, respectively. hsCRP levels were measured by using an immunoassay for hsCRP with a validated, high-sensitivity assay with an autoanalyzer (IMMAGE Immunochemistry Systems, Beckman Coulter, California). Its analytic sensitivity (lowest measurable CRP concentration that can be distinguished from zero) is 0.2 mg/L.

Interventional procedure

Coronary balloon angioplasty and stenting procedures were performed using low osmolar, non-ionic contrast agents (iopromidum) with standard percutaneous techniques by highly experienced operators. Intravenous bolus of unfractionated heparin (100 IU/kg) was administered to all patients. No additional bolus of heparin or glycoprotein IIb/IIIa inhibitors was infused during or after the procedure. The sheath was removed immediately after the end of the procedure. The operator was not blind to the treatment.

ECG monitoring

A 12-lead ECG was recorded before and 1 h after PCI and on the following day. During the procedure, three ECG leads were constantly monitored. Occurrence, severity and duration of chest pain, acute ST segment elevation or depression (0.1 mV) and/or T-wave abnormalities were recorded. Peri-procedural variables such as length and diameter of the stent and duration of the inflation were recorded. For at least 24 hours, patients were monitored for new-onset chest pain and ECG changes including ST-segment (elevation or depression (0.1 mV) from baseline), T-wave changes and emergence of pathological Q waves. Pathological Q waves were defined as those of at least 30 ms width and deeper than 25% of the correlating R amplitude, in at least two of the three diaphragmatic leads (II, III, aVF), in at least two of the four anteroseptal leads (V1-V4), or in at least two of the lateral leads (I, Vl, V5, V6).

Angiographic analysis

The cineangiograms were reviewed by two experienced angiographers who coded lesion-related morphological variables and were blind to the results of biochemical assays. Intimal major or minor dissection, thrombus, abrupt closure in a previously patent vessel, no reflow, spasm and side-branch occlusion were assessed. No reflow was defined as impaired or missed flow in the presence of an apparently open coronary vessel. Left ventricular function was assessed by angiography in all patients.

Statistical analysis

Analyses were performed using a SPSS software package (version 13.0 forWindows, SPSS Inc., Chicago, Illinois). The incidence of myonecrosis in the trimetazidine and standard groups were

Table 1. General and clinical features of both groups

compared using odds ratio (OR), and its associated 95% confidence interval (CI). Data are expressed as numbers and percentages for discrete variables and as means \pm S.D. for normally distributed continuous variables, and as median and interquartile range for other continuous variables. Results with a p value <0.05 were considered significant. Continuous variables were compared by Student's t test.

Results

General features, clinical, angiographic and PCI characteristics did not significantly differ between the two groups (p>0.05) (Table 1 and Table 2). The mean values of baseline CK-MB, troponin I and hsCRP levels did not also significantly differ between the two groups (p>0.05). Among the post-procedural mean values of CKMB and

	Group 1(n:59)	Group 2(n:60)	p value
Age (years)	53.8±8.80	52.4±8.78	0.38
Male sex	42(71%)	41(68%)	0.73
Current	2(0/44)	21(510/)	0.41
Smoking	20(%044)	31(31%)	0.41
Hypertension	29(%49)	38(63%)	0.12
Diabetes	21(520/)	29(620/)	0.22
Mellitus	31(32%)	38(03%)	0.23
Prior	10(200/)	20(220/)	0.71
Myocardial Inf.	18(29%)	20(33%)	0.71
Total Cholesterol	1(0+40	175 - 40	0.45
(mg/dl)	169±40	1/5±48	0.45
HDL	22 (7:0.00	22 70 7 7 70	0.04
(mg/dl)	33.67±8.90	33./9±7.70	0.94
LDL	102 27 21 40	110 40 25 76	0.10
(mg/dl)	102.2/±31.49	110.40±35.76	0.19
Triglyceride	1(2,04)0(,01	1(0,00+102.((0.7(
(mg/dl)	163.84±86.91	169.08±102.66	0.76
Medication			
Beta-blocker	57(96.6%)	57(95%)	0.75
ACE-I/ARB	39(66.1%)	38(63.3%)	0.39
ASA	50(84.7%)	54(90%)	0.66
Statin	33(55.9%)	31(51.7%)	0.64
Calcium Channel	12(0/20.2)	10(16 79/)	0.60
Blockers	12(7020.3)	10(10./%)	0.00
Clopidogrel	10(%16.9)	10(16.7%)	0.96

Values are mean \pm S.D. or n (%), p < 0.05: statistically significant

TnI levels, the values at the 24.hour were found to be the highest, and taken in to account for comparison with the pre-procedural values. The target arteries were successfully opened in all patients in both groups.

The pre-procedural mean values of CKMB levels in group 1 and group 2 were 3.42 ± 1.24 , 3.40 ± 1.20 , respectively (p>0.05). Post-procedural mean values of CK-MB levels at 6., 12. and 24. hours were found to be 3.90 ± 1.15 , 4.58 ± 1.19 , 6.17 ± 2.13 (the highest value), respectively in group 1 while the same values were found to be 3.80 ± 1.88 , 6.95 ± 3.14 , 11.30 ± 4.26 (the highest value), respectively in group 2 (control group). The rise in the mean value of CKMB (Δ CKMB) within 24 hours were significantly higher in group 2 compared to group 1. (7.90 vs 2.75, p<0.0001)

The pre-procedural mean values of troponin I levels in group 1 and group 2 were 0.24 ± 0.29 and



Figure 1. Comparison of variation of CK-MB values between the groups (Group 1: Trimetazidine , Group 2:Control)

0.23±0.29, respectively (p>0.05). Post-procedural mean values of troponin I levels at 6., 12. and 24. hours were 0.57±0.34, 0.70±0.29, 0.90±0.27 (the highest value), respectively in group 1 while the same values were 0.63±0.31, 0.82±0.24, 0.99±0.24 (the highest value), respectively in group 2. The rise in the mean value of troponin I (Δ troponin I) within 24 hours were insignificantly higher in group 2 compared to group 1. (0.75 vs 0.72, p>0.05)

The pre-procedural mean values of hsCRP levels in group 1 and group 2 were 0.46 ± 0.24 and 0.49 ± 0.27 , respectively (p> 0.05). Post-procedural mean value of hsCRP level at the 24. hour was 1.58 mg/L in group 1 while the same value was 8.69 mg/L in group 2. The rise in the mean value of hsCRP (Δ hsCRP) at the 24. hour was significantly higher in group 2 than in group 1 (8.2 vs 1.12 p<0.0001)



Figure 2. Comparison of variation of troponin I levels between the groups (Group 1: Trimetazidine, Group 2:Control)

	Group 1(n:59)	Group 2(n:60)	p value
Multivessel	14(22,70/)	18(200/)	0.44
stenting (n,%)	14(23.7%)	18(30%)	0.44
Drug-eluting stent	12(220/)	17(28,29/)	0.42
using (n,%)	13(2270)	17(28.576)	0.43
Number of stent			
implanted (n,%)			
1	47 (79.6%)	48 (80%)	
2	10 (16.9%)	11 (18.3%)	
3	2 (3.38%)	1 (1.67%)	
Stent (n,%)	36(61%)	34(56.7%)	0.61
Balloon (n,%)	3(5%)	3(5%)	0.97
Stent+Balloon	20(22,00/)	22(28,59/)	0.66
(n,%)	20(33.9%)	23(38.5%)	0.00

Table 2. PCI characteristics of patients in both groups

There was no myocardial infarction (detected by clinical findings, ECG changes), recurrent ischaemia requiring urgent target lesion revascularization or death in none of the groups during the in-hospital and 2 months follow-up periods.

Discussion

Percutaneous coronary intervention (PCI) has been one of the most sophisticated revascularization methods in cardiology. Creatine kinase (CK) or CK-MB isoenzyme (CK-MB) may elevate in 5% to 30% of patients undergoing PCI⁴ indicating the association between PCI and myocardial damage. PCI-related myocardial damage may be due to side-branch occlusion, vasospasm and dissection in complicated cases, and may be due to micro-embolization, reperfusion injury caused by baloon deflation, and minor side-branch occlusion in uncomplicated cases as well. In other terms, the major mechanisms underlying the PCI-related damage are acute ischemia and reperfusion injury. Reperfusion injury is largely attributable to increased levels of oxidative stress⁵ comprising FOR produced by the injured mitocondria and by the neutrophils accumulated in the reperfused myocardial territory. FOR primarily induces lipid peroxidation leading to inhibition of protein synthesis. The subsequent catastropic events leading to cellular death constitutes inactivation or degradation of numerous enzymes and cellular swelling accompanied by accumulation of intracellular calcium. The extent of reperfusion injury largely depends on the duration of ischemia before the reperfusion. Therefore, the duration and total number of baloon inflation have been considered as major determinants of PCI-related myocardial reperfusion injury. The shorter duration of balon inflation has been suggested to be associated with higher degrees of myocardial reperfusion injury possibly as a result of blunted ischemic pre-conditioning. Direct stenting without predilation has been regarded as a safe and advantageous procedure in selected cases⁶. In the present study, the majority of patients in both groups underwent direct stent implantation. The procedures in both groups did not lead to any complications including vasospasm, major side branch occlusion or dissection.

A lot of agents including adenosine7, glycoprotein 2b-3a receptor antagonists8, statins9 and naproxen¹⁰ have been tried in an effort to overcome the PCI-related myocardial injury. Besides these agents, trimethazidine (trimethoxybenzyl piperazine) (TMZ) has also been tried due to its effects on myocardial energy metabolism. Long chain fatty acid oxidation (FAO) is the primary metabolic pathway for myocardial energy production (ATP). Proper functioning of the heart depends on glycolysis only to a lesser extent¹¹. FAO, besides producing less amounts of ATP, also triggers anaerobic glycolytic pathway via inhibiting aerobic glycolysis (due to inhibition of pyruvate dehydrogenase, a major enzyme in aerobic glycolysis). Therefore, inhibition of FAO and thereby shifting to aerobic glycolysis produces considerably higher amouts of ATP and elevates the ischemia thresold in patients with coronary artery disease (CAD). TMZ is an effective antianginal agent 12 , and acts via inhibition of long-chain FAO. TMZ selectively inhibits long chain 3-keto acyl-coenzyme A thiolase (3-KAT), the last enzymatic step in the long chain FAO¹²⁻¹⁴. Amelioration of mitocondrial oxidative metabolism by TMZ administration as determined by increased 99mTcsestamibi uptake was previously demonstrated in the hibernating myocardium¹⁵. TMZ was also shown to reduce intracellular calcium accumulation and acidocis^{16,17}, and was shown to harbour potent endogenous anti-oxidant features^{18,19}. Therefore, TMZ may have the potential to improve both ischemic and reperfusion injury (as in the event of PCI-related myocardial damage).

Some previous studies have also demonstrated the beneficial effects of TMZ on myocardium in patients undergoing PCI. Kober et al.²⁰ demonstrated the acute significant effects of intracoronary TMZ administration (6 mg) on the improvement of electrocardiographic ischemic changes due to balllon inflation during PCI. Bonello et al.²¹ demonstrated significant reduction in the PCI-related myocardial infarction due to the preprocedural oral TMZ loading (60 mg) in patients undergoing PCI. Kuralay et al.²² demonstrated the suppressive effects of TMZ (begun 3 days before PCI) on inflammatory markers including TNF alpha and CRP in patients undergoing PCI. Labrau et al.²³ propounded TMZ (starting 15 days before PCI and continuing for 3 months after PCI) as a beneficial agent for minimizing PCI-related reperfusion injury and for the improvement of ventricular wall motion on transthoracic echocardiography (TTE) in a population of patients with acute coronary syndromes undergoing PCI.

In the present study, pre-procedural TMZ administration (starting 1 week before PCI) was found to reduce PCI-related myocardial damage in a population who underwent uncomplicated PCI. Compared with the previous studies, the loading dose was not administered, and the patients enrolled did not have acute coronary syndromes but stable angina pectoris. The control group (no trimetazidine given) was found to have significant rises in the mean values of CKMB and hsCRP, and insignificant rise in the mean value of troponin I while the trimethazidine group had no post-PCI increases in the mean values of these markers indicating a trimethazidine associated myocardial protection. The absence of PCI-related complications might have prevented further significant increases in the troponin I values in the control group.

On short term clinical follow-up (for 2 months), there was no myocardial infarction, need for revascularization or sudden cardiac death in none of the groups. Previous studies have particularly focused on the adverse prognosis in patients with significant CKMB and troponin I increases after PCIs. In the present study, due to the study design, long term (beyond 2 months) follow-up could not be performed, therefore it is not possible to suggest a trimethazidine- associated improvement in the long-term clinical outcome of patients undergoing PCI. There were also some limitations of the present study: the first one, as above mentioned, was the absence of long term follow-up of patients. The second limitation was the absence of any direct method used to demonstrate the postprocedural left ventricular function and myocardial viability.

Conclusion

In conclusion, the present study clearly demonstrated the protective effects of pre-procedural TMZ administration on myocardium in patients undergoing elective uncomplicated PCI. Future prospective randomized studies are still warranted to examine the effects of TMZ on clinical outcomes and survival in patients undergoing PCI.

References

- 1. Klein LW, Kramer BL, Howard E and Lesch M. Incidence and clinical significance of transient creatine kinase elevations and the diagnosis of non-Q wave myocardial infarction associated with coronary angioplasty. J Am Coll Cardiol 1991; 17: 621–626.
- 2. Abdelmeguid, AE, Topol EJ, Whitlow PL, Sapp SK and Ellis SG. Significance of mild transient release of creatine kinase-MB fraction after percutaneous coronary interventions. Circulation 1996; 94; 1528–1536.
- 3. Harris BM, Nageh T, Marsden JT, Thomas MR, Sherwood RA. Comparison of cardiac Troponin T and I and CK-MB for the detection of minor myocardial damage during interventional cardiac procedures. Ann Clin Biochem. 2000; 37 (Pt 6):764-769.
- 4. Califf RM, Abdelmeguid AE, Kuntz RE, Popma JJ, Davidson CJ, Cohen EA, Kleiman NS, et al. Myonecrosis after revascularization procedures. J Am Coll Cardiol1998; 31: 241–251.
- 5. Dhalla NS, Elmoselhi AB, Hata T, Makino N. Status of myocardial antioxidants in ischemia-reperfusion injury. Cardiovasc Res 2000; 47: 446-456.
- 6. Briguori C, Sheiban I, De Gregorio J, Anzuini A, Montorfano M, Pagnotta P, Marsico F, et al. Direct coronary stenting without predilation. J Am Coll Cardiol 1999; 34: 1910-1915.
- 7. Lee CH, Low A, Tai BC, Co M, Chan MY, Lim J, Lim YT, et al. Pretreatment with intracoronary adenosine reduces the incidence of myonecrosis after non-urgent percutaneous coronary intervention: a prospective randomized study. Eur Heart J. 2007; 28(1): 19-25.
- 8. Anderson KM, Califf RM, Stone GW, Neumann FJ, Montalescot G, Miller DP, Ferguson JJ 3rd, et al. Long-term mortality benefit with abciximab in patients undergoing percutaneous coronary intervention. J Am Coll Cardiol 2001;37:2059–65
- 9. Pasceri V, Patti G, Nusca A, Pristipino C, Richichi G, Di Sciascio G; ARMYDA Investigators. Randomized trial of atorvastatin for reduction of myocardial damage during coronary intervention. Results from the ARMYDA (Atorvastatin for Reduction of MYocardial Damage during Angioplasty) study. Circulation 2004;110:674–8.

- 10. Ozdol C, Gulec S, Rahimov U, Atmaca Y, Turhan S, Erol C. Naproxen treatment prevents periprocedural inflammatory response but not myocardial injury after percutaneous coronary intervention. Thromb Res. 2007;119(4):453-9.
- 11. Stanley WC, Lopaschuk GD, McCormack JG. Regulation of energy substrate metabolism in the diabetic heart. Cardiovasc Res. 1997; 34(1): 25-33.
- 12. Kantor PF, Lucien A, Kozak R, Lopaschuk GD. The antianginal drug trimetazidine shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long-chain 3-ketoacyl coenzyme A thiolase.Circ Res 2000;86:580– 588.
- *13. Veitch K, Maisin L, Hue L. Trimetazidine effects on the damage to mitochondrial functions caused by is-chemia and reperfusion. Am J Cardiol 1995; 76:25B–30B.*
- De Maison L, Fantini E, Sentex E, Grynberg A, Athias P. Trimetazidine:in vitro influence on heart mitochondrial function. Am J Cardiol 1995;76:31B–37B
- 15. Ciavolella M, Greco C, Tavolaro R, Tanzilli G, Scopinaro F, Campa PP. Acute oral trimetazidine administration in-creases resting technetium 99m sestamibi Uptake in hibernating myocardium. J Nucl Cardiol 1998;5:128–133.
- 16. Kay L, Finelli C, Aussedat J, Guarnieri C, Rossi A. Improvement of long-term preservation of the isolated arrested rat heart by trimetazidine: effects on the energy state and mitochondrial function. Am J Cardiol. 1995; 76(6): 45B-49B.
- 17. Renaud JF. Internal pH, Na+ and Ca2+ regulation by trimetazidine during cardiac cell acidosis. Cardiovasc Drugs Ther. 1988; 1(6): 677–686.
- Maridonneau-Parini I, Harpey C. Effect of trimetazidine on membrane damage induced by oxygen free radicals in human red cells. Br J Clin Pharmacol. 1985; 20(2):148-151.
- 19. Singh D, Chopra K. Effect of trimetazidine on renal ischemia/reperfusion injury in rats. Pharmacol Res 2004; 50: 623-629.
- 20. Kober G, Buck T, Sievert H, Vallbracht C. Myocardial protection during percutaneous transluminal coronary angioplasty: effects of trimetazidine. Eur Heart J. 1992 Aug;13(8):1109-15.

- Bonello L, Sbragia P, Amabile N, Com O, Pierre SV, Levy S, Paganelli F. Protective effect of an acute oral loading dose of trimetazidine on myocardial injury following percutaneous coronary intervention. Heart. 2007; 93(6):703-7.
- 22. Kuralay F, Altekin E, Yazlar AS, Onvural B, Goldeli O. Suppression of angioplasty-related inflammation by pre-procedural treatment with trimetazidine.. Tohoku J Exp Med. 2006; 208(3): 203-12
- 23. Labrou A, Giannoglou G, Zioutas D, Fragakis N, Katsaris G, Louridas G. Trimetazidine administration minimizes myocardial damage and improves left ventricular function after percutaneous coronary intervention.. Am J Cardiovasc Drugs. 2007;7(2):143-50.

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Effect of Trimetazidine treatment on left ventriculer functions and NT-ProBNP levels in patients with ischemic heart failure: Preliminary report

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Abstract

Objective: Metabolic therapy offers concrete help in the management of coronary artery disease and heart failure. Trimetazidine is a pharmacological agent that shifts the preference for energy substrate away from fatty acid metabolism and towards glucose metabolism. The purpose of this study was to investigate the changes in plasma N-terminal pro–B-type natriuretic peptide (NTproBNP) levels, changes in left ventricul (LV) systolic and diastolic functions, and changes in symptoms and exercise tolerance during a threemonth therapy with trimetazidine in patients with ischemic LV dysfunction.

Study design: The study included 33 patients (2 females, 31 males; mean age 62 ± 9 years) with ischemic LV dysfunction. All patients had sinus rhythm and resting ejection fraction <50%. In addition to their conventional treatment, trimetazidine was initiated with a dose of 20 mg t.i.d., which continued for at least 3 months. Blood samples for plasma NTproBNP were obtained and transthoracic echocardiography and exercise stress tests were performed at enrollment and after three months.

Results: After three months, significant improvements were detected in effort dyspnea (p<0.05), effort angina (p<0.05), LV end systolic volume (p<0.001), end systolic diameter (p<0.01), ejection fraction (p<0.001), myocardial performance index (p<0.05), global wall motion score index (p<0.001), septal annular systolic myocardial velocity (p<0.05) and maximum metabolic equi-

valent parameters (p<0.005). No significant improvement was detected in LV diastolic function parameters as no significant reduction achieved on natriuretic peptide levels.

Conclusion: Trimetazidine improves exercise tolerance and LV systolic functions in patients with ischemic LV dysfunction

Keywords: Ischemic heart failure, trimetazidine, NT-proBNP.

Introduction

The continuous of the pathophysiological background of heart failure is fundamental in the management of this complex disease. Although there are considerable modern therapeutic advences, heart failure remains a leading cause of morbidity and mortality in developed and increasingly in developing countries with a 5-year mortality rate of approximalitely %50. The prognosis worsens with the advancement of heart failure and mortality rate in patients in New York Heart Association (NYHA) class IV ia as high as 50% per year.¹ Early neurohumoral activation with sympathetic overdrive interplaying with progressive hemodynamic changes constitutes the main characteristic of heart failure independent of its etiology.² Neurohormone activation is characteristic of HF, and elevation of circulating N-terminal pro-brain natriuretic peptide (NT-pro-BNP) levels, and other neurohormones is directly releated to mortality and morbidity. ³⁻⁴ In addition, plasma NT- pro -BNP, secreted mainly from ventricle, has been known as a useful prognostic indicator in patients with HF. 5-6

Trimetazidine (TMZ) is a piperazine derivative (1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride) with anti-ischaemic properties. It is the first in a new class of metabolic agents, available for clinical use. In conditions of hypoxia or induced ischaemia, TMZ maintains homeostasis and cellular functions by selectively inhibiting 3-ketoacyl-CoA-thiolase.⁷ As a consequence, fatty acid b-oxidation is reduced and glucose oxidation is stimulated, resulting in decreased cellular acidosis and higher ATP production.8-9 In humans, TMZ has been shown to increase the ischaemic threshold and to relieve angina pectoris in patients with coronary artery disease. These benefits have been observed without any change in heart rate, blood pressure, and rate-pressure product at rest, during submaximal and peak exercise.¹⁰⁻¹¹ More recently, TMZ improved the mechanical efficiency of dysfunctional myocardium in patients with ischaemic cardiomyopathy. This effect was associated with enhanced left ventricular diastolic filling and systolic function. There is also demonstration that TMZ has antioxidant properties. During acute and chronic ischaemia, TMZ reduces the loss of intracellular potassium (K) induced by oxygen free radicals and also the membrane content of peroxidated lipids.¹²

The aim of the present study is to deternine the effects of TMZ, administered as an adjuant to standart therapy in patients with ischemic cardiomyopathy, on left ventriculer function and N therminal pro BNP (NT proBNP) levels.

Patients and methods

Thirty-six patients with heart failure and New York Heart Association (NYHA) functional class II or III who were already receiving optimal heart failure treatment consisting of angiotensinogenconverting enzyme inhibitors or angiotensin receptor blockers, carvedilol, spironolactone, digitalis and furosemide were included in the study. The etiology of the heart failure was coronary artery disease documented by coronary angiography in all patients. Following baseline echocardiographic examination, trimetazidine, at a dose of 20 mg three times/day, was added to the therapy and patients were reevaluated 3 months later. Exclusion criteria were; NYHA class IV, acute myocardial infarction in the previous 3 months, atrial fibrillation, LV ejection fraction (LVEF)> 50%, severe valvular heart disease, alcoholic cardiomyopathy, pacemakers, renal failure (serum creatinine>2.0 mg/dL), chronic lung disease or any systemic disorder and use of additional drugs such as antiarrhythmics. The study was approved by hospital Ethics Committee and patients gave their written informed consent.

Blood sampling and laboratory determinations

Blood was taken at the same visit as the clinical and echocardiographic assessment. Following at least 15 min of recumbency, blood sampling was undertaken. Blood specimens were cool centrifuged immediately, and the plasma stored at -30 C without freezethaw cycles until measured. The samples were analysed using the Roche Diagnostics NT- proBNP kit on a Modular Analytics E170. The results are given in pg/mL. The clinician who provided follow-up fort he patients and performed the echocardiographic measurements was blinded to NT-pro BNP results. The measurement was performed by employing the electrochemiluminescence immunoassay principle. The test was calibrated to detect levels 5pg/mL and 35.000 pg/mL.

Echocardiography methods

All patients were examined lying on their left side and images were digitally obtained (GE Vivid 7 Ultrasound). Echocardiographic measurements were performed according to the recommendations of the American Socitey of Echocardiography (ASE).¹³ A full clinical echocardiogram was performed including: parasternal M-mode recordings; biplane Simpson's left ventricular volumes and ejection fraction (EF); mitral valve pulsed wave Doppler (PWD); pulmonary venous PWD and isovolumic relaxation time (IVRT). Mitral valve inflow PWD was recorded with the sample volume between the leaflet tips and then recorded again during the Valsalva manoeuvre. IVRT recordings were made with a PWD sample volume placed in the LV outflow tract. All echocardiographic images were obtained according to a standardized protocol by specially trained research sonographers, without knowledge of the patients'

clinical details. In addition to the standard techniques described, tissue PWD was also performed by placing a 5 mm sample volume on the medial and lateral aspects of the mitral valve annulus. The signal was optimized and recorded at 100 mm/s sweep speed. The average of both measurements was used. None of the patients had haemodynamically significant valve disease.

Echocardiographic measurements

Triplicate measurements were made according to standard methods and included: (i) two-dimensional and m-mode measurements: left ventricular end-diastolic dimension (LVEDD), left ventricular endsystolic dimension, left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV); (ii) Doppler measurements: mitral valve peak early filling velocity (E), peak late filling velocity (A), deceleration time (DT) of the mitral E wave, A wave duration (Adur), IVRT; pulmonary venous peak systolic velocity (S), peak diastolic velocity (D), atrial reversal velocity (AR), atrial reversal duration (ARdur); (iii) tissue Doppler measurements: mean of lateral and medial mitral annular E velocity (Ea), mitral annular A velocity (Aa), mitral annular S velocity (Sa). The following variables were calculated: E:A, ARdur-Adur, stroke volume (SV)=LVEDV-LVESV, ejection fraction (EF)=SV/LVEDV x 100%; E/Ea. The estimated pulmonary capillary wedge pressure (PCWP) eas calculated by using following verified Formula:14 PCWP=17+(5XE/A)-(0.11XIVRT) and PCWP=1.9+1.24XE/E'. Myocardial Performance Index (MPI) was calculated by subtracting the ejection time from the total systolic time and by dividing this value by the ejection time.

Statistical analysis

Statistics were obtained using the ready-to-use program of SPSS version 13.0. Categorical variables are reported as frequency and group percentages. Continuous variables are presented as means \pm S.D. The McNemar test was utilized to compare the dependent variables. The Wilcoxon test was used to compare the dependent variables optanied before

Table 1. Baseline characteristics of study patients (n=33)

Clinical features	
Age (years) (means±SD)	62±9
Males (%)	94
BMI (kg/m ²) (means±SD)	26±3.3
Hypertension (%)	61
Diabetes Mellitus (%)	30
Smoking (%)	9
Hyperlipidemia (%)	97
Coronary artery disease	100
Medical treatments	
ACE-I (%)	63
ARB (%)	18
Diuretics (%)	30
Digoxin (%)	9
Spironolactone (%)	15
Beta blockers (%)	66
Statin (%)	64
Nitrates (%)	9

Table 2. Hemodynamic, laboratory parameters and functional class before and after trimetazidine treatment

	pretreatment	Post-treatment	p value
Systolic BP (mmHg)	131±20	128±16	0,5
Diastolic BP (mmHg)	78±10	75±8	0,1
Heart rate (beat/min)	75±11	79±11	0,009
Glucose (mg/dl)	109±25	116±39	0,17
HbA1c (µIU/ml)	6,9±1	7,1±1,2	0,5
Hb (g/dl)	14±1,1	14±1,2	0,9
NT-proBNP (pg/ml)	400±430	352±396	0,054
Uric acid (mg/dl)	5,7±1,4	5,7±1,3	0,9
NYHA Class			
Class I (%)	82	88	0,5
Class 2 (%)	18	12	0,5

Data are expressed as means±SD, NYHA: New York Heart Association, Hb: Hemoglobin, NT-pro BNP: N-terminal pro–B-type natriuretic peptide

and after measurements. The correlation among the study variables were examined by Pearson's correlation coeffcient. P<0.05 was accepted as statistically significant.

Results

The study was completed by 33 patients, as 1 patient died before the completion of the study, 1 patient underwent bypass surgery, and 1 patient

was excluded due to irregular use of the study drug. Table 1 shows the baseline clinical characteristics of the study patients. There was no need to change the conventional therapy of our patients during the study period. The patients' hemodynamic, laboratory parameters and funcional class are presented in Table 2. After 3 months, there was no difference in blood pressure or BMI, whereas a significant increase in heart rate was seen. During the study period, the metabolic parameters were

Table 3. Effects of trimetazidine on left ventricular echocardiographic parameters

	pretreatment	Post-treatment	p value
LVEDV (cm ³)	139±57	132±55	0,07
LVESV (cm ³)	84±37	76±39	0,001
LVEDD (mm)	55±7	54±7	0.06
LVESD (mm)	42±7	40±8	0,002
EF (%)	40	44	0,001
MPI	0.93±0.35	0.91±0.4	0.046
Wall motion score index	1.92±0.47	1.75±0.5	0,001
	61±15.6	62±2.2	0,26
E (cm/s)	72±16.3	70±16	0,5
A (cm/s)	198±54	209±56	0,22
EDT (ms)	147±33	146±38	0,87
IVRT (ms)	101±26	100±26	0,92
ADT (ms)	53±14.6	52±11.5	0,61
PVS (cm/s)	52±15	51±17.3	0,9
PVD (cm/s)	34±8	32±7	0,46
PVrA (cm/s)	106±36	109±30	0,23
tPVrA (cm/s)	114±19	110±17	0,38
tA (ms)	-7.94±35	-0.52±34	0,29
$\Delta t (PVrA-A) (ms)$			
S' cm/s	6.4±1.6	6.9±1.5	0,02
lateral	7.6±2	7.1±1.4	0,14
septal			
E' cm/s	6.4±1.8	6.1±1.6	0,26
lateral	9±2.5	9.1±2.9	0,98
septal			
A' cm/s	8.6±1.8	9.1±2.2	0,32
lateral	10.5±2.1	10.6±2.2	0,93
septal			
E/E'	10±3	10.6±3.1	0,19
lateral	7.1±2.4	7.4±2.8	0,36
septal	8.6±2.6	9±2.7	0,27
Mean	12.5±3.1	13.1±3.3	0,27
Estimated PCWP (mmHg)			

LVEDV: left ventricular end diastolic volume; LVESV: left ventricular end systolic volume; LVEDD: left ventricular and diastolic diameter; LVESD: left ventricular end systolic diameter; EF: ejection fraction; MPI: myocardial performance index, EDT: E-wave deceleration time; IVRT: isovolumetric relaxation time; ADT: A-wave deceleration time; PVS: peak pulmonary venous systolic wave velocity; PVA: peak pulmonary venous diastolic wave velocity; PVrA: peak pulmonary venous reverse A-wave time, PCWP: pulmonary capillary wedge pressure

well controlled, with no pronounced differences. We observed a trend of decreasing NT-proBNP levels (p=0.054).

Left ventriculer end-sistolic volüme (LFESV), Left ventriculer end-diastolic volüme (LVESD), and wall motion score index significantly decreased in comparison to baseline, while ejection fraction (EF) significantly increased (p<0.05). LVEDV and LVEDD decreased slightly, but these decreases statistically significant. No significant difference in diastolic function parameters was observed between the groups. There was no significant difference in TDI parameters with the exception of annular systolic myocardial velocity (S' septal). S' septal improved significantly after 3-month therapy. However, the changes in LV diastolic inflow pressure parameters were equivalent between the two groups after treatment for 3 months (Table 3). Correlations Table 4 shows the correlations between echocardiographic and biochemical parameters in our sample. Significant correlations were observed between NT-proBNP and LVEDV, LVESV, wall motion score index, EF and PCWP (Table 4). In addition, the association found between Edt and E/A, PVD, IVRT before the treatment disappeared at three months.

Discussion

In the present study, significant improvements were observed in the symptoms and LV systolic functions of patients with ischemic cardiomyopathy after 3 months of trimetazidine therapy. The NT-proBNP level of the patients decreased, but the difference was not statistically significant. No difference was observed in diastolic function parameters following the 3 months of therapy. The NT-proBNP level, LV systolic functions and estimated filling pressures were significantly correlated. Activation of the neurohormonal system is the most critical mechanism in the progression of heart failure. Natriuretic hormone measurement has been reported to be beneficial in screening and classifying heart failure, determining prognosis, monitoring patients, and avoiding or limiting irrelevant and costly examinations.¹⁵ Similarly, reduced natriuretic hormone concentrations can be observed in patients undergoing beta blocker and ACE inhibitor therapy.¹⁶ Standard heart failure therapy has been demonstrated to lead to a reduction of 35%-50% in NT-proBNP and BNP levels.¹⁷⁻¹⁸ Some studies suggest that the clinical diagnosis can be more precise than BNP measurements in establishing asymptomatic LV dysfunction. The diagnostic value of BNP depends on the classification of LV dysfunction.¹⁹ Of NYHA class II heart failure patients, 21% were shown to have BNP level within the normal range.²⁰ Our study group consisted of patients with mild LV systolic dysfunction, with asymptomatic or mild symptoms. Two studies have investigated the effect of trimetazidine on BNP level. In the study of Fragasso et al.²¹ 55 patients with heart failure were treated with trimetazidine for 13 months, and a significant decrease was observed in BNP level. The baseline functional capacity of the patients in the study conducted by Fragasso et al. ²¹ was class II or III, with a mean EF < 35%. Of the heart failure patients, 36% were non-ischemic. Fragasso et al. ²¹ reported a decrease of 49% in the mean BNP level; however, the BNP level was not established for all patients. In the present study, we also observed a reduction of 12% in natriuretic peptide level. However, our study differs from that of Fragasso et al.²¹ in terms of the clinical characteristics of our patients. Furthermore, the functional class of our patients was lower, and the duration of our study was shorter. We also included patients with ischemic LV dysfunction. The lack of statistical significance of the decrease in NT-proBNP level in our study could be attributed to the impact of the time elapsed from the onset of LV dysfunction and to the limited number of patients. Dinapoli et al.²² observed a significant decrease in BNP level after 6 months of trimetazidine therapy in a group of patients with severe heart failure. Brottier et al.²³ observed improvements in symptoms and EF in the 6-month trimetazidine group compared to a placebo group among patients with severe ischemic cardiomyopathy and a LV EF < 40%, and they demonstrated that the effect continued up to the 12th month. Vitale et al.²⁴ observed an increase of 18% in EF after 6 months of trimetazidine therapy and a significant decrease in end-systolic and end-diastolic diameters and volumes. However, that study significantly differed from the present study not only with respect to the mean age (78 years) and the inclusion of patients with advanced

heart failure symptoms and findings, but also with respect to the therapy that the patients were administered (95% diuretic and 80% digoxin vs. 30% diuretic and 9% digoxin in our group). Consistent with that study, an increase of 10% in the EF and a significant decrease in end-systolic diameters and volumes in mild heart failure patients was observed in our study after 3 months of therapy. The increased EF (%20-23) achieved in studies was not inferior to the increase reported in a number of revascularization studies.²⁴ These results suggest that trimetazidine increases LV systolic function independent of age and severity of heart failure. However in the present study, only about half of the patients were complaining of significant dyspnea before entering the study and this could also explain the relatively low levels of pro-BNP.

This is also evidenced by the fact that 82% of the patients were in NYHA class I before entering the study.

Along with LV systolic function, alterations in diastolic filling are generally associated with the clinical presentation of heart failure.25 Although it is not clearly understood, decreased stroke volume and increased LV filling pressure in response to exercise are considered responsible for poor exercise tolerance.²⁶ Vitale et al.²⁴ observed better diastolic function following 6 months of trimetazidine therapy. They based their conclusion on an increased E/A ratio and a significant decrease in Adt, IVRT, and PVS waves. In the present study, we observed an insignificant increase in E/A ratio and DT, while the Adt, IVRT, and PVS waves remained the same. The differences in patient characteristics and follow-up periods could be responsible for the inconsistency between the two studies.

NT-proBNP was correlated with LV systolic function parameters in the present study, but no correlation was observed with LV diastolic function parameters. A decrease of 59% has been reported in the E/A ratio after 6 months of trimetazidine therapy combined with beta blockers in a study involving patients with advanced–functional class heart failure.²⁷ However, these patients were known to have a restrictive filling pattern as well as a shorter DT and IVRT. In addition, the velocity of the reverse A wave, a pulmonary vein wave, increased in delayed relaxation and false normal

patterns. ²⁶ The mean PVrA-wave amplitude and the E/A ratio of our study group are consistent with a delayed relaxation pattern. No difference was observed in IVRT values.

While a DT value < 150 msec nearly always indicates an increase in the mean left atrial pressure and a longer DT, it is generally associated with a left atrial pressure < 15 mmHg. A short DT has been reported to be associated with a poor prognosis.²⁶ An increase in the E/A ratio is the most common parameter utilized to evaluate improvement in diastolic function. We observed a slight increase in the E/A ratio in response to 3 months of therapy, but the difference was not statistically significant.

Progressive systolic and diastolic abnormalities lead to a progressive reduction in TDI velocities.²⁸ In the evaluation of LV systolic function, TDI-derived annular systolic myocardial velocity (S) is an important parameter.²⁹ A strong correlation was noted between septal S and EF in patients with heart failure. In another study, no alterations were noted in TDI parameters after a month of trimetazidine therapy in 20 diabetic patients (mean age, 66 years) with ischemic cardiomyopathy who had class II–III functional capacity.³⁰ Similarly, no differences were observed in TDI parameters after 3 months of therapy in the present study.

The most reliable non-invasive method to establish LV filling pressure is the E/E' ratio, and a strong correlation has been found between E/E' and pulmonary capillary wedge pressure (PCWP) and cardiac catheterization parameters.³¹ A mean E/E' ratio > 9 has been correlated with a LV end-diastolic pressure > 15 mmHg [27]. Here, the mean E/E' was 8.6±2.6, and the estimated PCWP was 12.5±3.1. Our results are consistent with previous reports.

BNP is an independent marker of increased LV end-diastolic pressure and is correlated with functional capacity and prognosis.³² The diagnostic accuracy of BNP for PCWP and LV filling pressure in advanced heart failure patients is reported to be 74%.³³⁻³⁴ Consistent with the literature, the NT-proBNP level was significantly correlated with the estimated PCWP and mean E/E' ratios in the present study. After 3 months of trimetazidine therapy, no differences were noted in the E/E' or estimated PCWP. However, a lack of a significant response to therapy could be attributed to the fact that our study sample consisted of clinically stable patients.

Study limitations

The short follow-up time, limited number of patients and lack of a control group were the limitations of our study. In addition, the small number of female patients was a major constraint of the present study. The non consecutive recruitment may explain the very low percentage of female gender. Furthermore, invasive methods such as catheterization were not employed in the assessment of LV function. However, the sensitivity and specificity of echocardiographic Doppler measurements have been reported to be adequate in a number of studies. Because an echocardiographic evaluation is an observer-dependent method and inter-observer variance is a major factor, the scientific reliability of our results may be diminished. Finally, a survival analysis was not conducted.

Conclusion

Our findings support the use of trimetazidine in ischemic cardiomyopathy. Although the beneficial effects of trimetazidine on prognostic factors in heart failure have been revealed in a number of studies, its effect on prognosis has not been clearly demonstrated. Long-term prospective studies with larger study samples with similar characteristics are needed to investigate its effect on prognosis.

References

- 1. Pericle Di Napoli, Alfonso A Taccardi: ''Trimetazidine: the future of cardiac function'' Future Cardiol 2009:5(5);421-24
- 2. Frenneaux MP. Autonomic changes in patients with heart failure and in post-myocardial infarction patients. Heart. 2004;90(11):1248-55
- 3. Koglin J, Pehlivanli S, Schwaiblmair M, Vogeser M, Cremer P, vonScheidt W. Role of brain natriuretic peptide in risk stratification of pa-tients with congestive heart failure. J Am Coll Cardiol 2001;38:1934-41
- 4. Kim SH, Kim JS, Baek KK, et al. Role of NT-proBNP in evaluation of functional status in congestive heart failure. Korean Circ J 2004;34: 894-9.

- 5. Yasue H, Yoshimura M, Sumida H, et al. Localization and mechanism of secretion of NT-pro-natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. Circulation 1994;90:195-203.
- 6. Tsutamoto T, Wada A, Maeda K, et al. Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure: prognostic role of plasma brain natriuretic peptide concentration in pa-tients with chronic symptomatic left ventricular dysfunction. Circulation 1997;96:509-16.
- 7. Kantor PF, Lucien A, Kozak R, Lopaschuk GD. The antianginal drug trimetazidine shifts cardiac energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial long-chain 3-ketoacyl coenzyme A tiolase. Circ Res 2000;86:580–588
- 8. Harpey C, Clauser P, Labrid C, Freyria JL, Poirier JP. Trimetazidine, a cellular anti-ischemic agent. Cardiovasc Drug Rev 1989;6:292 312
- 9. Stanley WC, Lopaschuck GD, Hall JL, Mccormack JG. Regulation of myocardial carbohydrate metabolism under normal and ischaemic conditions: potential for pharmacological interventions. Cardiovasc Res 1997;33:243–257
- 10. Detry JM, Sellier P, Pennaforte S, Cokkinos D, Dargie H, Mathes P. Trimetazidine: a new concept in the treatment of angina. Comparison with propranolol in patients with stable angina. Br J Clin Pharmacol 1994; 37:279–288.
- 11. Szwed H, Hradec J, Preda I. Anti-ischaemic efficacy and tolerability of trimetazidine administered to patients with angina pectoris: results of three studies. Coron Artery Dis 2001;12(Suppl. 1):S25–S28
- 12. Belardinelli R, Solenghi M, Volpe L, Purcaro A. Trimetazidine improves endothelial dysfunction in chronic heart failure: an antioxidant effect. ,Eur Heart J. 2007 May;28(9):1102-8
- Schiller NB, Shah PM, Crawford M, DeMaria A, Devereux R, Feigenbaum H, et al. Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. American Society of Echocardiography Committee on Standards, Subcommittee on Quantitation of Two-Dimensional Echocardiograms. J Am Soc Echocardiogr: 1989;2(5):358-67.
- 14. Pirat B, Zoghbi W. Echocardiographic assessement of left ventriculer diastolic dysfunction. Anadolu Kardiyol Derg. 2007;7(3):310-5.
- 15. Clerico A, Emdin M. Diagnostic accuracy and prognostic relevance of the measurement of cardiac natriuretic peptides: a review. Clin Chem 2004; 50:33-50.
- 16. Clerico A, Fontana M, Zyw L, Passino C, Emdin M. Comparison of the diagnostic accuracy of brain natriuretic peptide (BNP) and the N-terminal part of the propeptide of BNP immunoassays in chronic and

acute heart failure: a systematic review. Clin Chem 2007; 53:813-822.

- O'Hanlon R, O'Shea P, Ledwidge M, O'Loughlin C, Lange S, Conlon C, et al. The biologic variability of B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide in stable heart failure patients. J Card Fail 2007; 13:50-55.
- Zugck C, Haunstetter A, Krüger C, Kell R, Schellberg D, Kübler W, et al. Impact of beta-blocker treatment on the prognostic value of currently used risk predictors in congestive heart failure. J Am Coll Cardiol 2002; 39:1615-1622.
- 19. Cosson S. Usefulness of B-type natriuretic peptide (BNP) as a screen for left ventricular abnormalities in diabetes mellitus. Diabetes Metab 2004; 30:381-386.
- 20. Godkar D, Bachu K, Dave B, Niranjan S, Khanna A. B-type natriuretic peptide (BNP) and proBNP: role of emerging markers to guide therapy and determine prognosis in cardiovascular disorders. Am J Ther 2008; 15:150-156.
- Fragasso G, Palloshi A, Puccetti P, Silipigni C, Rossodivita A, Pala M, et al. A randomized clinical trial of trimetazidine, a partial free fatty acid oxidation inhibitor, in patients with heart failure. J Am Coll Cardiol 2006; 48:992-998.
- 22. Di Napoli P, Di Giovanni P, Gaeta MA, D'Apolito G, Barsotti A. Beneficial effects of trimetazidine treatment on exercise tolerance and B-type natriuretic peptide and troponin T plasma levels in patients with stable ischemic cardiomyopathy. Am Heart J 2007; 154:602.e1-5.
- 23. Brottier L, Barat JL, Combe C, Boussens B, Bonnet J, Bricaud H. Therapeutic value of a cardioprotective agent in patients with severe ischaemic cardiomyopathy. Eur Heart J 1990; 11:207-212.
- 24. Vitale C, Wajngaten M, Sposato B, Gebara O, Rossini P, Fini M, et al. Trimetazidine improves left ventricular function and quality of life in elderly patients with coronary artery disease. Eur Heart J 2004; 25:1814-1821.
- 25. Vom Dahl J, Altehoefer C, Sheehan FH, Buechin P, Uebis R, Messmer BJ, et al. Recovery of regional left ventricular dysfunction after coronary revascularization. Impact of myocardial viability assessed by nuclear imaging and vessel patency at follow-up angiography. J Am Coll Cardiol 1996; 28:948-958.
- 26. Parthenakis FI, Kanakaraki MK, Kanoupakis EM, Skalidis EI, Diakakis GF, Filippou OK, et al. Value of Doppler index combining systolic and diastolic myocardial performance in predicting cardiopulmonary exercise capacity in patients with congestive heart failure: effects of dobutamine. Chest 2002; 121:1935-1941.

- 27. Düzenli MA, Özdemir K, Aygül N, Zengin K, Gök H. The role of tissue Doppler echocardiography in the evaluation of functional capacity of patients with heart failure. Turk Kardiyol Dern Ars 2008; 36:143-149.
- 28. Fedorova TA, Il'ina IuV, Sotnikova TI, Rybakova MK. Potentialities of cytoprotection in the treatment of chronic heart failure in patients with coronary heart disease. Klin Med 2004; 82:15-20.
- 29. Baykan M, Yilmaz R, Celik S, Orem C, Kaplan S, Erdol C. Assessment of left ventricular systolic and diastolic function by Doppler tissue imaging in patients with preinfarction angina. J Am Soc Echocardiogr 2003; 16:1024-1030.
- Mornos C, Cozma D, Rusinaru D, Ionac A, Maximov D, Petrescu L, et al. A novel index combining diastolic and systolic Tissue Doppler parameters ort he non-invasive assessment of left ventricular enddiastolic pressure. Int J Cardiol 2009;136(2):120-9.
- Thrainsdottir IS, von Bibra H, Malmberg K, Rydén L. Effects of trimetazidine on left ventricular function in patients with type 2 diabetes and heart failure. J Cardiovasc Pharmacol 2004; 44:101-108.
- 32. Lim HS, Kang SJ, Choi JH, Ahn SG, Choi BJ, Choi SY, et al. Is E/E' reliable in patients with regional wall motion abnormalities to estimate left ventricular filling pressure? Int J Cardiovasc Imaging 2009; 25:33-39.
- 33. Epshteyn V, Morrison K, Krishnaswamy P, Kazanegra R, Clopton P, Mudaliar S, et al. Utility of B-type natriuretic peptide (BNP) as a screen for left ventricular dysfunction in patients with diabetes. Diabetes Care 2003; 26:2081-2087.
- 34. Parsonage WA, Galbraith AJ, Koerbin GL, Potter JM. Value of B-type natriuretic peptide for identifying significantly elevated pulmonary artery wedge pressure in patients treated for established chronic heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 2005; 95:883-885

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Esophageal cancer in north of Iran: The effect of p53 codon 72 polymorphism

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Abstract

Introduction: The p53 gene is one of the most extensively studied human genes because of its role as a tumor suppressor. Its diverts functions include DNA binding, cell cycle control, DNA repair, differentiation, genomic plasticity, and apoptosis. A common polymorphism of the p53 gene at codon 72 has been associated with human cancer susceptibility and prognosis. P53 mutations are seen in all kinds of hystologic cancers .In this study, we investigated codon 72 polymorphism in northern Iran.

Method: This was a cohort study including forty esophagus cancer cases and 40 healthy individual as control group.AS-PCR method was applied for determination of codon 72 polymorphism.

Results: Among 40 patients, there were 4 (%10) homozygote Arg/Arg ,22 (%55) heterozygote Arg/ pro and 14 (%35) homozygote prolin. In control group, 8 (%20) from 40 samples were homozygote Arg/Arg, 24 (%60) hetrozygote prolin Arg and 8 (%20) hemozygote prolin.The distribution of genotypes in esophagus cancer cases and controls were statistically significant (P=0.001).

Conclusion: According to the results the Pro allel has an elevated frequency among patients. The different distribution of codon 72 genotypes in patients is a result of biochemical difference of two form of p53 (one with Arg at codon 72 and the other with pro). Arg form of P53 is a stronger apoptosis inducer; however this form is more vulnerable to proteasomic degradation. On the other hand, though Pro form of P53 is a strong transcriptional factor, it is a weaker apoptosis unducer. Generally according to the examined population of patients, Allele pro codon 72 had more frequency ,that represents its probable role in induction esophageal cancer in northern Iran.

Key words: p53 codon 72, esophageal cancer, Iran.

Introduction

Cancer of the esophagus is the eighth most common cancer worldwide with more than 400.000 cases per year incidence [1-2].The two main types of esophageal cancer are squamous cell carcinoma and adenocarcinoma [3]. The cause of esophageal cancer is unknown, How ever , epidemiologic studies in several areas of the world suggest a relationship between some factors like Alcohol, Tobacco, Nitrosamine, Vitamin deficiencies, Aflatoxin, Candidal, viral infectious and this malignancy [4-6].

The incidence of esophageal cancer shows certain geographic variation from South African countries to the other regions in a way that Iran, china, India, Ceylon and Puerto- Rico are considered as high incidence areas [7-9]. Another important charactristics of this disease is that early esophageal cancer may not cause [10]. Diagnosis in people without symptom is rare, and usually accidental because of tests done to check other medical problems. Unfortunately, most esophageal cancer does not cause symptoms until they have reached an advanced stages, when acure is less likely. In addition, Many risk factors play a role in the etiology of esophageal cancer, although these vary with geographic region. For example, betel chewing and oral consumption of opium are factors primarily found in southeast Asia and the Caspian sea area [8]. Whereas P53 as transcription factor, has a role of diagnosing inner and outer signals and indeed has the role of keeping stability of hereditary material [11]. Generally each factor that can change in DNA, causes the cell cycle arrest and reparation of the damage or apoptosis. Destroying the activity of p53 is very current in carcinogen process and it seems that it is regarded as a prerequisite for this stage. Making mutation in p53 is observed in more than 50% of all human cancers [10]. Today there are more than 2000 scientific essay that introduce mutation in p53 in wide groups of cancers as the most current genetic changes [12]. This gene has 10 known polymorphisms. One of these polymorphisms that has been studied a lot, exists in codon 72. This polymorphism causes producing two forms of p53 molecule which are in 72 codon have Arg or pro [13]. frequency of allele gene p53 in codon 72 in different population and in different geographical areas is variable[14]. Considering all stated above, the aim of this study was to examine P53 codon 72 polymorphism on esophageal cancer in north of Iran.

Material and Method

This was a case control study conducted on the total of 40 tumor biopsies plus 40 samples of the control groups in north of Iran.biopsy specimens were collected from operation theatre of gastro endoscopies in the internal department of Shahid Rajai Tonekabon hospital. This samples has been collected from 2008 to 2011. Tissue samples were stored in -20 -70 degree centigrade.

Filtering DNA from samples (by fermentase kit)

First prior to DNA extraction, the sample should be digested for a night with digestion buffer 100ml and 2.5ml proteinase k.Mixing binding solution with tissue samples has been carried out with the ratio 1 to 3 (100ml to 300ml),and then 5ml of silica was added.Ancubation was carried out for 5 miniutes in the temperature of 55° c.washing buffer was added to the settle and Then vortex was performed .the quick centrifusion 3 times, for 5-10 seconds was performed and then DNA was extracted during this process and the result was analysed in Agarose gel 0.8 %.



Figure 1. Extracted DNA in gel Agarose %0.8 column M, in related to molecular weight marker, and the other columns with numbers 1-8 are related to samples

P53 polymorphism analysis

Exon 4 of the p53 Gene containing the poly morphic sequence variant at codon 72 was analysed using direct genomic sequencing. The following primers have been used:

- Arg- primer F:5' –

TCCCCCTTGCCGTCCCAA-3' (25 Pmol) - Arg- primer R:5' – CTGGTGCAGGGGGCCACGC-3' (25 Pmol) - Pro- Primer F: 5'- GCCA-GAGGCTGCTCCCCC- 3' (25 Pmol) - Pro- Primer R: 5'- GCCA-GAGGCTGCTCCCCC- 3' (25 Pmol)

Results

In this study, 40 esophageal cancer pations and 40 Normal Biopsys for control group have been selected. The samples were kept in -20 °C – 70 °C.All the samples as mentioned in the last part, have been DNA extracted and then the result has been analysed in gel Agaroz 0.8%.

The use of molecule weight marker, was due to making sure of the suitable quality of extracted DNA for PCR. As observed in figure 1, the amount of extracted DNA fragility is little .

Detection of codon 72 genotype, was done by AS-PCR method. As mentioned already in part 2 pairs of primers for doing PCR, were used. Two pairs of primers that are designed for Allels Arg/



Figure 2. PCR codon 72 production in Agarose gel 2% M column is for molecular weight marker. Each sample was examined once for Arg and again for pro so that the genotype of each sample to be specified, as it is with R and P for the samples 1 to 3. So the sample 1 has the genotype of RP and the sample 2 has the genotype of RR and the third sample has the genotype of PP

pro, just in the case of existence of sequence with the related Allel, make the production of required segment. For prolin it is equals 177 bp, and for Allel Arg it equals to 141 bp (Figure 2).

Other findings showed that from 40 pations, there were 4 (%10) cases of homozygote Arg/Arg ,22 (%55) cases of heterozygote Arg/pro and 14 (%35) cases of homozygote prolin. On the other hand, from 40 samples of control group, ther were 8 (%20) cases of homozygote Arg/Arg, 24 (%60) cases of hetrozygote prolin Arg and 8 (%20) cases of hemozygote prolin. The distribution of genotypes in esophagus cancer cases and controls were statistically significant (P=0.001).

Discussion

Cancer of esophagus is the eighth most common cancer world wide with more than 400000 case per year incidence [1,2]. The cause of esophageal cancer in unknown, However epidemiologic studies in several areas of the world suggest its relationship with alcohol, tobacco, nitrosamine vitamin deficiencies aflatoxin, candidal and viral infections [4-6]. Esophageal cancer incidence increase with age – specific rate were very low below 50 year of age . In compare with women, men have a 3 fold higher rate of esophageal cancer .Diets full of fruits and vegetables are linked to a lower risk of esophageal cancer. In another word, about %15 of esophageal cancers can be linked to a diet poor in fruits and vegetables, in the way that daily diet is considered much more important than environmental factors [15].

Genetic factors have also an important role in this malignancy.Genetic changes cause increasing sensitivity to some environmental factors. Among the genes, P53 having the main role in induction cancer. Generally each factor that cause changes in DNA can activate P53 and cell cycle arrest and reparation of damage or apoptosis.

Destroying the activity of P53 is very current in carcinogen process and seems that it is regarded as a prerequisite for this stage.Making mutation in p53 is observed in more than %50 of all human cancers [14]. Moreover P53 mutations are seem in all kinds of histological cancers like colon cancer (%60),stomach cancer (%60)lung cancer (%20),brain cancer (%48) esophagus cancer (%60) [16]. One of these polymorphism that has been studied a lot, exists in codon 72. This polymorphism causes producing two forms of p53 molecule which are in 72 codon have Arg or pro [17]. frequency of allele gene p53 in codon 72 in different population and in different geographical areas is variable[13].

According to the results of this study, the various rate between Alleles Arg and pro has been showed moreover prolin allele has higher frequency in esophageal cancer in northern Iran that they all represent the effect of this protein on esophageal cancer as well as the results of other studies regarding P53 in malignant esophagus tumors [16-17].

Reference

- 1. Parkin DM, Pisahi P, Ferlayj. Estimates of the world wide incidence of 25 major cancers in 199. Int j cancer 1999; 8:827_41.
- Ghadimi MR, MahmoodiM, Mohammad K, RasouliM, ZeraatiH, FotouhiF, Factors affecting survival of patients with oesophagealcancer: a study using inverse Gaussian frailty models, Singapore Med J ,2012; 53(5): 336
- 3. Forman .Review article: oeso phago_gast rice.adeno carcinoma_an epidemiological perspective.Aaliment phar macol. There 2004; 20:55_60.
- 4. Boyce HW .Tumours of the Esophagus.In sleisenger MH.F ford tru j s(eds):gatroin tostinal diseas.5 edietion (volume) philadelphia. W B sander. 1993. P: 401-18.
- 5. Osmanog UN.Disease of the esophagus in llicinc G.unal S.Biberoglu K. Akalins.suleymanlar G (EDS):Basic medicine. 1 st edition (volume 1).Ankara.Gunes kitaberi 1996.p:941-9(in Turkish)
- 6. Boyce HW .Tumours of the Esophagus.In sleisenger MH.F ford tru j s(eds):gatroin tostinal disease.5 edietion (volume) philadelphia. W B sander. 1993. P: 401-18.
- 7. Dumont p, leu JI, dellapietra AC [1], Georg, DL and murphy M:the codon 72 polymorphic variants of p53 have markedly diferent apoptotic potential.Nut genet 33:357-365,2003
- Hed, zhang DK, lam KY, Mal, Ngan HY, Liuss, T sao SW.prevalence of hpv infection in esophageal squamus cell carcinoma in Chinese. Patients and its relationship to the p53 gene mutation.aiant jcancer 1997; 72:959-964

- 9. chen B, Yin H, Dhuran dher N.Detection of human papiloma-virus DNA in esophageal squamous cell larcinoma by the polymerase chain reaction using general consensus primers, Hum pathol 1994;25:920-923
- 10. Eloubeidi MA, Desmond R, Arguedas MR, Reed CE, Wilcox CM.Prognostic factors for the survival of patients with esophageal carcinomain the US: the importance of tumor length and lymph node status. Cancer 2002; 95:1434-43.
- 11. StankovicV, MitrovicS, Jancic S, KnezevicM, Azanjac G, TanaskovicI et al. Correlation of p53 expression levels with the degree of histological differentiation histological stages of colorectal carcinomas, Healthmed Journal, 2012;6(1)
- 12. Chang F.syrjanen s. shen Qet al.Human papilloma virus (HPV) DNA in esophageal precancer lesions and squamus cell carcinomas from china int.]Cancer: 45:21-5-1990
- 13. Hollstein M, Rice K, Greenblatt MS et al. Database of p53 gene somatic ~ mutations in human tumors and cell lines. Nucleic Acids Res;2000; 22:3551.
- 14. G. Orth, F. Breitburd, M. Favre, and O. Croissant: Papillomaviruses. Possible role in human cancer. In H. H. Hiatt, 1. D. Watson, and J. A. Winsten, eds.: Origins of Human Cancer. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory,2000: 1043-1068.
- 15. Schwarz, E., Freese, U.K., Gissmann, L., Mayer, W., Roggenbuck, B., Strernlau, A., and zur Hausen, H. Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. Nature,2000; 314: 111-114
- 16. Pickens et al., Human esophageal cancer molecular genetics, apoptosis signalling pathway esophageal vanser thrapeutics, 2003
- 17. Kleinberg LR,et al cancer of esophagus.In :Abel off MD,et al.Abeioffs clinical oncology.4th ed.philade churchill living stone ,2008:1399

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Evaluation of doctoral nursing programs by PhD students: A Perspective from Turkey

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Abstract

Introduction/**Aim:** Evaluation of doctoral nursing programs is very important to generate data for program improvement. Especially student's evaluation of the education process should be essential part of the continuous effort to increase and assure quality in the higher education. Therefore, the purpose of this study was to examine the current state and issues of doctoral nursing programs in Turkey from the perspective of recipients of the educational process.

Methods: An Health Science Institute, with nine doctoral nursing programs and 61 students, was target setting. Fifty three doctoral nursing students participated to sample of this study. Data were collected a semi-structured interview form that consisted of questions about opinions and recommendations of doctoral nursing students. The survey was conducted using self-administered survey technique. The Program SPSS, version 16.0 for Windows was used for the statistical data processing.

Results: Most of the participants stated that, contents of courses met their expectations from doctoral education (52.8%), courses had also included actual information related to issues (73.6%), number of credits in doctoral training program was sufficient (86.8%), and stages of doctoral courses provided/should have provided background for their studies of thesis (50.9%). However, the majority of participants (54.7%) believed that stages of doctoral course didn't provide/wouldn't provide adequate statistical background for their thesis.

Conclusion: Most of doctoral nursing students had positive perceptions about their doctoral education programs, except statistical background for their studies of thesis.

Key words: Doctoral education in nursing, nursing science, Doctor of Philosophy (PhD)

Introduction

Doctoral education in nursing is necessary for the development of nursing science; it prepares students to advance their discipline of nursing science through research and theory development, to contribute to the body of nursing knowledge, and to provide leadership to the profession (Erdil and Bayraktar, 2001; Lash, 2009; Ozsoy, 2007). So, the rapid increase in doctoral nursing programs and as a result evoluations quality of doctoral nursing programs are a worldwide trend (Nagata et al. 2012). In Turkey, doctoral education in nursing is carried out with Doctor of Philosophy in Nursing (PhD). PhD programs intend to allow students realize new discoveries in science, to develop new scientific methods and apply these to new areas, and thus to be trained as integrating and propagating scientists (www.istanbul.edu.tr). These programs are also based on patient care, too (Yavuz, 2004).

In Turkey, doctoral degree in nursing program started as a single nursing program in 1972 (Aksayan and Cimete, 2000; Bahcecik and Alpar, 2009). Currently, there are nine different programs (Fundamentals of Nursing, Medical Nursing, Surgical Nursing, Women's Health and Obstetrics Nursing, Children's Health and Disease Nursing, Mental Health and Psychiatry Nursing, Public Health Nursing, Administration in Nursing and Education in Nursing), and there exist eleven schools of nursing at public universities offering doctorate degree in nursing (Can, 2010; Thobaben et al., 2005; Akpınar ve Şengün 2011). However, according to recent Turkish Higher Education Council (HEC-Yuksekogrenim Kurumu-YOK) rules, at least 5 academics (1 full professor) are needed to start a PHD program. Therefore, some doctoral nursing programs had to be closed. A doctoral program consists of a course period, proficiency

exam and followed by thesis work that lasts over 4 years (Orer, 2011). To be admitted to these programs, all applicants have to accomplish Graduate Record Examination, and foreign language test. Also, Grade Point Average (GPA) must be achieved in undergraduate and Master's programs (Platin, 2002; Yavuz, 2004). After being admitted to a program a student takes basic course units, and a seminar unit. Then, student takes the proficiency exam that is separated as written and oral. That exam is evaluated by Doctorate Proficiency Committee (Yavuz, 2004). After completing the proficiency exam, student selects a dissertation topic. A doctoral student generally has one advisor who is familiar with the topic (Bahcecik and Alpar, 2009; Can, 2010).

All doctoral nursing programs controlled by the HEC. The Council of Research and Development in Nursing (HEMAR-G) has constituted a "National Commission of Nursing Doctorate Education" (Kocaman, 2009). As the PHD is an international degree, as it comprises national/international collaboration and cooperation, researcher mobility, it must meet the international criteria, besides the national commission standards.

In the past, nursing schools and universities have evaluated doctoral programs with their own criteria, but global quality criteria are necessary for evaluating doctoral programs. The international committee of International Network for Doctoral Education in Nursing developed global quality criteria, standards and indicators (QCSI) for the evaluation of doctoral programs in nursing and it has been considerably used around the world (IN-DEN, 2004). In this QCSI, the major criteria were: the nature of the mission, quality of the faculty, standards for admission, progression and graduation, composition of the curriculum, program administration, and resources (Miki et al., 2011; Arimoto et al., 2012). ORPHEUS (Organisation for PhD Education in Biomedicine and Health Sciences in the European System) explained the need for harmonization and evaluation quality of PhD programs in biomedicine and health sciences and set some standards regarding research environment, admission policy and criteria, PhD training program, supervision PhD thesis, assessment PhD thesis, structure of the institution (ORPHE-US, 2012). Evaluation of doctoral programs provides data for program improvement, and assess the adequacy of resources to carry out the program's mission (Kim et al., 2006). To enhance the quality of doctoral programs in nursing, the students, graduates, and faculty members should be involved in program evaluation (Kjellgren et al., 2005; Miki et al., 2011). Especially student's evaluation of the education process should be essential part of the continuous effort to increase and assure quality in the higher education (Cervinka et al., 2011). However, in the literature, evaluating doctoral education is anchored mostly in voices of faculty and administrators (Miki et al., 2011). Nevertheless, there are a few studies which is drawn on students' perspectives. Objective of this study is to improve the quality of the doctoral education by evaluating doctoral students' perspectives and shape the future of nursing doctoral education. Because, increase in the number of researchers, developing nursing knowledge and basing nursing applications on scientific knowledge are all related with the quality of PhD programs.

Material and methods

Setting and Sample

This descriptive research was carried out in the University of Istanbul Florence Nightingale School of Nursing which is one of the leader schools providing nursing doctoral degree. Florence Nightingale School of Nursing keeps up nursing doctoral degree programs since 1987. Today, Florence Nightingale School of Nursing provides several nursing doctoral programs and there are so many nursing doctoral students coming from various universities in Turkey for nursing doctoral education. At 2009-2010 academic year, when this study conducted, there were 61 doctoral nursing students in Florence Nightingale School of Nursing in Istanbul University, Health Science Institute. Fifty three doctoral nursing students participated the study, voluntarily. Response rate was 86.8%.

Data Collection

The data were collected with a semi-structured questionnaire form. The questionnaire was formed according to national and international literature as open-ended and close-ended questions. Students' reasons for doctoral education and perspectives regarding phases of doctoral education programs (characteristics of courses, proficiency exam, dissertation phase, etc) were questioned with 22 close-ended questions. Students' recommendations regarding doctoral education and vocational training/academic plans were questioned as openended questions. In addition the questionnaire included demographic information (age, marital status, occupational experience as a nurse or as an academician) and questions about previous nursing education and phase of doctoral education. Face validity for questionnaire was determined by researchers. This self-administered questionnaire was completed within 20-25 minutes.

Data Analysis

Data regarding with closed-ended questions were analyzed by using the SPSS program (version 16.0) or windows and, were evaluated statistically percentage and mean values. Answers for open-ended questions were classified via thematic analysis method by assistance of four specialists' separately.

Ethical considerations

Permission to conduct this study was received from the school's educational committee which is the review board for research in education within the university. The doctoral students were informed about the purpose of the study and what would be expected of them. They were assured of their right of refusal to participate in or to withdraw from the study at any stage without any negative consequences. The anonymity and confidentiality of students was guaranteed.

Results

Average age of the doctoral students was 30±5.01, 52.8% single. Almost all participants

Table 1. Reasons for starting doctoral education*

(84.9%) had occupational experience as a nurse after bachelor's degree and most of them (55.2%) had 1-24 months experience. Majority of doctoral students (71.7%) were research assistant, and also a great percentage of them worked as research assistants in the same school they were attended for doctorate education, however 30.2% of them were assigned by other schools for doctorate education.

Almost half of the doctoral students stated that they hadn't selected occupation willingly (47.2%). Most of them (66%) completed bachelor's and master's programs in the same university. In addition, 83% of the students didn't change their branch of master's degree program in doctorate program. Great percentage of the students (62.3%) were in the dissertation study phase.

The majority (73.6%) of students were started doctoral education to gain more perspectives toward nursing science (Table 1).

Most of the participants believed that Academic Graduate Education Exam (45.3%), Foreign Language Exam (88.7%), Proficiency Exam (77.4%), Undergraduate Academic Average (71.7%) is absolutely necessary (Table 2).

About theoretical teaching methods, participants stated that "contents of courses meet the expectations from doctoral education" (52.8%), "courses also include actual information relating to issues" (73.6%), "teaching methods of courses facilitated my understanding of lessons "(41.5%), " number of credits in doctoral training program are sufficient" (86.8 %), "doctoral courses phase provides/will provide background for my study of thesis" (50.9%), "doctoral courses phase provides/will provide adequate practical background for my study of thesis" (54.7%). However, majority of students (54.7%) believed that doctoral course phase didn't provide/wouldn't provide adequate statistical background for their studies of thesis. When we look at perspectives regarding

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/ 1./
71.7
69.8
73.6
69.8

* multiple item selected

proficiency exam, majority of participants indicated that "proficiency exam is a test that measures the theoretical information" (56.6%) and "proficiency exam is necessary for passing to dissertation phase" (37.7%). Doctorate students, who were in dissertation process (n=33), stated that "I have determined my subject" (87.9%) and "my thesis advisor has determined my subject" (12.1%). In addition, they explained that their dissertation subjects included their interests (90.9%) and their dissertation subjects were related to their thesis advisors' interests (57.6%). Furthermore, most of the students (66.7%) stated that their dissertation subjects were not related to their subjects of master thesis. With respect to thesis advisors, 66.7 % of students expressed that their thesis advisors spared enough time for their dissertations and most of them (78.8 %) explained that their thesis

Opinions	Agree		Undecided		Disagree	
	n	%	n	%	n	%
Academic Admission Criterias of Doctoral Education						
Program						
Academic Graduate Education Exam is absolutely		15.0		15.0	•	0.7.7
necessarv	24	45.3	9	17.0	20	37.7
Foreign language exam is absolutely necessary	47	88.7	3	5.7	3	5.7
Proficiency exam is absolutely necessary	41	77.4	10	18.9	2	3.8
Academic average score of undergraduate education is	20	71.7	_		10	10.0
absolutely necessary	38	/1./	5	9.4	10	18.9
Opinions Regarding Education With Theoretical Methods						
Course contents meet the expectations for doctoral						
education	28	52.8	14	26.4	11	20.8
Courses also include actual information relating to issues	39	73.6	7	13.2	7	132
Teaching methods of courses facilitated my understanding		10.0	,	10.2	,	10.2
of lossons	22	41.5	12	22.6	19	35.8
Number of credits in Doctoral training program are						
construction of electrics in Doctorial training program are	46	86.8	6	11.3	1	1.9
sufficient						
Doctoral courses phase provides/will provide background	27	50.9	16	30.2	10	18.9
for my study of thesis					<u> </u>	
Doctoral courses phase provides/will provide adequate	7	13.2	17	32.1	29	54 7
statistical background for my study of thesis	,	13.2		52.1		54.7
Doctoral courses phase provides/will provide adequate	20	547	12	226	12	22.6
practical background for my study of thesis	29	54.7	12	22.0	12	22.0
Opinions Regarding Proficiency Exam						
Proficiency Exam is a test that measures the theoretical	20	566	10	10.0	12	245
information	30	30.0	10	18.9	13	24.3
Opinions Regarding Dissertation Phase			1	[
My thesis involves my interests	30	90.9	2	6.1	1	3.0
My thesis subject is related to my supervisor's interests	19	57.6	4	12.1	10	30.3
My thesis supervisor spared enough time for my thesis	22	66.7	7	21.2	4	12.1
My thesis supervisor supports me in scientific / academic	26	70.0	1	12.1	2	0.1
aspects	20	/8.8	4	12.1	3	9.1
My thesis will contribute to the development of nursing	30	90.9	2	6.1	1	3.0
Proficiency Exam is necessary for passing to disertation	20	27.7	16	20.2	17	
phase	20	51.1	16	30.2	1/	32.1

Table 2. Opinions Regarding Doctoral Education

advisors supported them for scientific/academic aspects. However, students said that they believed their dissertations should contribute to the improvement of nursing science (91.0%) (Table 2).

About most difficult parts of dissertation study (Table 3) students mostly reported the average of difficulty as; data collection (24.2 %), statistical analysis (15.1 %) and all parts (24.2 %).

Table 4 portrays results of the doctoral students' recommendations regarding doctoral education. Firstly, they recommended that doctoral courses' teaching methods should be changed (11.3%); secondly, they recommended that more opportunity should be given for experimental study designs and projects (7.5%); thirdly, they recommended that practical courses should be increased for theoretical and application integration (5.7%), research courses and statistics courses should be taken in the same period and should be integrated (5.7%) and doctoral students should take the-

ir courses from different faculty members from different fields/institutes (5.7%); fourthly, they recommended that doctoral students should be encouraged to do more publications and researches that are accompanied by advisors (3.8%); fifthly, they recommended that advisors should support the scientific development of doctoral students (1.9%), dissertation subject should be determined during the course phase (1.9%) and a student in a higher semester and more experienced, should be assigned to mentor for doctoral students, in departments (1.9%). After completing doctoral education, these nursing doctoral students' plans regarding vocational training /academic were mostly (50.9%) regarding to academic development.

Discussion

In Turkey, doctoral nursing programs have many challenges in Turkey. The foundation in which researches were executed is one of the le-

Table 3. Most diffucult parts of dissertation study (n= 33).

Parts of Dissertation	n	%
Selecting dissertation subject	3	9.2
Data collection	8	24.2
Statistical analysis	5	15.1
Writing	1	3.0
Selecting dissertation subjects and statistical analysis	3	9.2
Data collection and statistical analysis	2	6.1
Statistical analysis and writing	1	3.0
Data collection, statistical analysis and writing	1	3.0
All parts	8	24.2
Missing data	1	3.0

Recommendations	n	%
Course teaching methods should be changed	6	11,3
Doctoral students should be encouraged in order to do more publications and researchs that are accompanied by supervisors	2	3.8
Practical courses should be increased for theoretical and application integration	3	5.7
Supervisors should support the scientific development of doctoral students	2	3.8
Doctoral students should take their courses from different faculty members from different fields / institutes.	3	5.7
Dissertation subject should be determined during the course phase	1	1.9
In departments. a student, in a higher semester and more experienced, should be assigned to mentor for doctoral students	1	1.9
Research courses and statistics courses should be taken in the same period and integrated	3	5.7
More opportunity should be given for experimental study designs and projects	4	7.5

ader nursing schools providing nursing doctorate education in Turkey. It provides post graduate education to graduated nurses and some of them are working in this foundation and also in other foundations as a research assistant. Nursing students working in other foundations as a research assistant can be assigned for their post graduate education in this foundation. So this foundation comprises of various doctoral students and this study reflects a general perspective about doctoral education.

In this study, 30,2% of participants were assigned by other schools for doctorate education due to non-existence of nursing doctoral programs at the university they were being employed.

Most of the nursing doctoral students' started doctoral education to gain more perspectives toward nursing, to contribute to the improvement of nursing profession, to discuss and search about nursing issues and to sustain academic careers. Similarly, Jolley (2007) stated that nurses want to study for a doctoral degree because they want to search. Also, American Association of Colleges of Nursing AACN (2006) pointed out that doctorate programs (PhD) focus heavily on scientific content and research methodology and provide a scholarly approach to the discipline and a commitment to the advancement of the profession (www.aacn.edu.tr). Wellington and Sikes (2006) explained that real benefit of doctoral education are to gain knowledge, understanding and indeed the professional awareness and skills. In addition Jolley (2007) explained that doctoral education provides scientific and practical development about nursing. However, some of the nursing doctoral students' reasons for attending these programs, besides other reasons, was sustaining their academic careers (Canbulat et al. 2007). As in Turkey, there are inadequate doctoral programs and academician nurses, students primarily attend these programs for academic careers.

In this study most of the nursing doctoral students absolutely agreed on necessity of doctoral education program's academic admission criteria. Anderson (2000) stated that most nursing programs use the similar criteria for admission. With respect to theoretical methods of programs; nursing doctoral students stated that course contents, actual information relating to courses, teaching methods and number of credits of courses met their expectation. Similarly, Allen et al. (2002) found that PhD graduates were satisfied with their program; Miki et al. (2011) stated that, 80–90% of the graduate students highly rated that the program goal and curriculum were consistent with the philosophy and mission of the university.

However, in a study of the National Commission of Nursing Doctorate Education, most of the students (72.5%) agreed that nursing doctorate program content should have been rearranged (Kocaman 2009). Also, in Kacmaz's (2001) study nursing doctoral students explained that doctorate courses should not be like master studies, and the doctorate course content should be based on research in nursing. In addition, nursing doctoral students in our study agreed that doctoral course phase provided/ would provide theoretical and practical background for thesis studies. But most of them believed that doctoral course phase didn't/ wouldn't provide adequate statistical background for their thesis studies. Furthermore, most of the participants stated that they were difficulties about statistical issues, like data collecting, statistical analysis. Similarly, in the a study of the National Commission of Nursing Doctorate Education, only 25% of the students stated that they are adequate for improving as an independent researcher (Kocaman, 2009).

However, doctorate students typically are required to take courses in a cognate for providing them the substantive content and models in order to assist them in the design of their research (Jolley, 2007, Kjellgren et al., 2005).

Most of the participants explained that their dissertation subjects were related to their thesis advisors' interests. Similarly, in International Network for Doctoral Education in Nursing (IN-DEN) quality criteria, standards and indicators for doctoral programs in nursing (2004) was emphasized that students' objectives for research had to be congruent with faculty members' research expertise and scholarship (INDEN, 2004). Most of the participants expressed that their thesis advisors supported them for in scientific/ academic aspects, as well. As Sambrook et al. (2008) stated, purpose of supervision is to steer, guide and support students through the process of conducting a doctorate. Therefore, the advisor must have sufficient social and technical skills to deal with the doctoral research. Another important subject about thesis advisors is availability. In this study most of the participants explained that their thesis advisors spared enough time for their thesis. But Sambrook et al. (2008) like Easterby-Smith and colleagues (2003) noticed that thesis advisor could be busy and spare less time for supervision. Thus one of the important criterions for doctoral advisor might be "available time" (Evans, 2007; Sambrook et al., 2008).

Most of participants expressed that their thesis would contribute to the development of nursing. Also, there is a general agreement in the National Institute of Nursing Research that the primary focus of nursing research should be clinical research that builds the scientific base of nursing practice (Anderson, 2000; Ellis, 2005).

Some of the participants suggested that doctoral students should have been encouraged to do more publications and research accompanied by advisors. Similarly Anderson (2000) emphasized that one of the important measures of quality in doctoral education was the opportunity for students to participate with faculty on research projects. According to the "Bologna Process" PhD programmes form the "third cycle" of higher education, following the Bachelor and Master cycles as a tool to develop a "knowledge society". However, the core component of the third cycle is the advancement of learning through original research, which makes the third cycle unique and different from the first and second cycles. In particular, PhD programmes are based primarily upon the PhD student doing original, hands-on research (ORPHEUS, 2012).

Another recommendation participants suggested was practical course should have been increased for integration of theory and practice. Because Anderson (2000) stated, many doctoral students did not mesh their clinical preparation and experience with their research. So when they finished, they found themselves in a situation in which their teaching and research were in different areas.

Some of the participants also suggested that doctoral students should have taken courses from different faculty members from different fields/ institutes. Similarly, in INDEN Quality Criteria, Standards and Indicators (QCSI) for doctoral programs in nursing (2004) also suggested to establish a pattern of productive scholarship, collaborating with researches in nursing and other disciplines in scientific endeavors. (www.indeen.org, Kim et al., 2006). However, Anderson (2000) and Kim et al. (2009) found that both providers and receivers identified interdisciplinary courses as strength because they thought the interdisciplinary courses provided an opportunity to link nursing with other disciplines. Other recommendation suggested by participants was mentoring from senior students. Similarly mentoring support found to be important for gaining appropriate scientific roles and research productivity (Kjellgren, 2005).

Conclusion

The majority of participant's reason for starting doctoral education was to gain more perspectives toward nursing. The majority of participants absolutely agreed about necessity of academic admission criteria's. About theoretical methods of the program, nursing doctoral student indicated that course contents, actual information relating to courses, teaching methods and number of credits of courses met their expectations. Although participants expressed that the doctoral course phase provided practical and theoretical background for their thesis studies, they also stated that this phase didn't provide adequate statistical background for their thesis studies. Participants thought that their dissertation would contribute to the development of nursing. And they also stated that their advisors provided enough support to them. Doctorate student should take adequate statistical courses, which will provide to help research project and dissertation thesis.

References

- 1. Akpinar, B., Şengün, F. Examination of the content of doctoral programs in nursing. 6th ORPHEUS Conference Book. 2011; 84.
- 2. Aksayan, S., G. Cimete.. Nursing education and practice in Turkey. Journal of Nursing Scholarship. 2000;32(2): 211-212.
- 3. Allen, C.M., E.M. Smyth, Wahlstrom, M. Responding to the field and to the academy: Ontario's evolving PhD. Higher Education Research & Development. 2002; 21: 203–214.

- American Association of Colleges of Nursing (AACN), (2006). The essentials of doctoral education for advanced nursing practice. www.aacn.nche.edu/IDC. pdf. (Retrieved from 04.04.2010).
- 5. Anderson, C.A. Current strengths and limitations of doctoral education in nursing: are prepared for the future?. J Prof Nurs. 2000; 16: 191-200.
- Arimoto, A., Gregg, M.F., Nagata, S., Miki, Y., Sachiyo, M. Evaluation of doctoral nursing programs in Japan by faculty members and their educational and research activities. Nurse Education Today.2012; 32:1-7.
- Bahcecik, N., Alpar, S.E. Nursing education in Turkey: from past to present. Nurse Education Today. 2009; 29: 698-703.
- 8. Can, G. Nursing education in Turkey. Nurse Educator. 2010; 35(4): 146-147.
- 9. Canbulat N. Demirgöz M. Cingil D. Saklı F. A general overview of the nursing academicians in Turkey. International Journal of Human Sciences. 2007; 4(1):1-8.
- 10. Cervinka, M., Cervinkova, Z., Mares, J., Hach, P. 6th ORPHEUS Conference Book. 2011;75.
- 11. Easterby-Smith, M., R. Thorpe,., Lowe, A. Management research: An introduction. 2nd edition, London: Sage Publications. 2003;15-16.
- 12. Ellis, L.B. Professional doctorates for nurses: Mapping provision and perceptions. Journal of Advanced Nursing. 2005; 50(4): 440–448.
- Erdil, F., Bayraktar, N. Hacettepe Universitesi'nde hemsirelikte lisans üstü eğitimin gelisimi [Development of the higher education of nursing in Hacettepe University]. 1st International and 5th National Nursing Education Congress Book. 2001;199-202.
- Evans, C. The experience of international doctoral education in nursing: An exploratory survey of staff and international nursing students in a British University. Nurse Education Today 2007;27(5): 499–505.
- 15. Istanbul Universitesi Saglık Bilimleri Enstitüsü [Istanbul University, Health Science Institute] http://www.istanbul.edu.tr/enstituler/saglik (Retrieved from 04.04.2011).
- International Network for Doctoral Education in Nursing, (2004). Quality Criteria, Standards, and Indicators (QCSI) for doctoral programs in nursing, www.umich.edu/~inden/quality/qsci1204.pdf. (Retrieved from 04.04.2011).
- 17. Jolley, J. Choose your doctorate. Journal of Clinical Nursing. 2007; 16(2): 225–233.

- Kacmaz, N. Hemşirelikte doktora eğitimi: Öğrenci gözüyle. [Doctorate education in nursing: students' view]. 1st International and 5th National Nursing Education Congress Book. 2001; 77-80.
- Kim, M.J., Lee, H., Kim, H.K., Ahn, Y.H., Kim, E., Yun, S.N., Lee, K.J. Quality of faculty, students, curriculum and resources for nursing doctoral education in Korea: A focus group study. International Journal of Nursing Studies. 2010; 47(3): 295–306.
- 20. Kim, M.J., McKenna, H.P., Ketefian, S. Global quality criteria, standards, and indicators for doctoral programs in nursing; literature review and guideline development. International Journal of Nursing Studies. 2006; 43(4): 477–489.
- Kjellgren, K.I., Welin, C., Danielson, E. Evaluation of doctoral nursing programs: A review and a strategy for follow up. Nurse Education Today. 2005; 25(4): 316–325.
- Kocaman, G. Türkiye'de Lisansüstü Hemşirelik Eğitiminin Durumu [Situation of nursing higher education in Turkey]. 12. National "International Attendance" Nursing Congress Book. 2009; 21-23.
- 23. Lash, A. Dünyada ve ülkemizde hemşirelikte lisansüstü eğitimin durumu ve gelişmeler. [Situation of nursing higher education in the world and in Turkey]. 12. National "International Attendance" Nursing Congress Book. 2009; 18-20.
- 24. Miki, Y., Gregg M.F., Arimoto A., Nagata, S., Murashima, S. Evaluation of doctoral nursing programs by doctoral students in Japan: Cross-sectional questionnaire survey. Japan Journal of Nursing Science. 2011;1-9, doi:10.1111/j.1742-7924.2011.00196.x
- Nagata, S., Gregg, M.F., Miki, Y., Arimoto, A., Murashima, S., Kim M.J. Evaluation of doctoral nursing education in Japan by students, graduates, and faculty: A comparative study based on a cross-sectional questionnaire survey. Nurse Education Today. 2011; 32:361–367.
- 26. Orer H.S. Implementation of ORPHEUS standards in Turkey. 6th ORPHEUS Conference Book. 2011; 23.
- 27. ORPHEUS/AMSE/WFME Task Force Standards for PhD Education in Biomedicine and Health Sciences in Europe. Aarhus University Press, Denmark, 2012.
- 28. Ozsoy, S.A. The struggle to develop nursing research in Turkey. International Nursing Review. 2007; 54(3): 243–248.
- 29. Platin, N. Doctoral Education in Nursing in The World and in Turkey.Doctorate Education Symposium, June 26, Dokuz Eylül University in Izmir, 2002.

- 30. Sambrook, S., Stewart, J., Roberts, C. Doctoral supervision . . . a view from above, below and the middle !. Journal of Further and Higher Education. 2008; 32(1): 71-84.
- Thobaben, M., Roberts, D.A., Badır, A. Exploring nursing education in the people's republic of China, Japan and Turkey. Contemporary Nurse. 2005;19(1-2): 15-16.
- 32. Wellington, J., Sikes, P. 'A doctorate in a tight compartment': Why do students choose a professional doctorate and what impact does it have on their personal and professional lives?. Studies in Higher Education. 2006; 31(6): 723–734.
- *33.* Yavuz, M. Nursing doctoral education in Turkey. Nurse Education Today. 2004; 24 (7): 553–559.
- 34. Yuksek Ogretim Kurumu (YOK) [Turkish Council of Higher Education]. (2011) www.yok.gov.tr. (Retrieved from 04.04.2011).

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Haptoglobin genotypes, inflammation status and their associations with hemoglobin levels in stage 4 chronic kidney disease patients with anemia

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Abstract

Background and aims: Anemia and increased inflammation are the main causes of mortality in chronic kidney disease (CKD) patients. This study was designed to explore the probable relationship of hemoglobin (Hb) with serum haptoglobin (HP), its genotypes and inflammation statues in CKD stage 4 patients with anemia.

Methods: The subjects of the study was 40 anemic CKD patients on stage 4 [GFR = 15 - 29 ml/ min/1.73 m²]. Serum biochemical factors as well as ferritin, HP and IL-6 levels were measured by standard methods. Haptoglobin genotypes were determent by PCR method and the accuracy of genotyping was confirmed by sequencing amplified fragments and RFLP analysis.

Results: Results showed that although HP2-2 was the most common genotype (%72.5), no significant correlations between HP genotypes with Hb and HP levels were found (p=0.49, p=0.32, respectively). Significant negative correlation between Hb level with IL-6 and hs-CRP levels (p<0.001 and p<0.05, respectively) and a significant positive correlation between Hb and ferritin levels was found (p<0.05).

Conclusion: The results suggest that HP genotype and serum HP levels have no significant effect on hemoglobin level in CKD stage 4 patients. However, Hb level is associated with inflammation and albumin status.

Keywords: Haptoglobin genotype, CKD, hemoglobin, inflammation.

Introduction

The incidence and prevalence of kidney disease worldwide has risen markedly in the past decade [1]. It has been shown that, the incidence of chronic kidney disease (CKD) in stages 3 to 5, in Iran is about 14.9% [2]. Chronic kidney disease is the most commonly defined by a reduction in kidney function [3]. The anemia is a common complication in patients with CKD and correlates significantly with the morbidity and mortality in these patients [4].

The main causes of anemia in CKD are reduced production of erythropoietin in the kidneys and erythropoietin resistance [5, 6]. Inflammation also has an important role in CKD complications [7]. Inflammatory factors such as IL-6 inhibit erythropoietin production, impair the growth of erythroblasts, decreases RBC survival and promote death of immature erythroblasts [5, 7]. Studies shown that patients who had higher plasma levels of inflammatory markers shows lower hemoglobin levels than those with lower level of inflammation [8]. On the other hand, Inflammation is an important factor associated with erythropoietin resistance and the occurrence of anemia in patients with CKD [9]. Inflammation also limits the availability of iron for erythropoiesis and expands the erythropoietin resistance [10]. One of the major inducible cytokine inflammatory proteins that participate in hemoglobin and iron metabolism called haptoglobin (HP) [11]. Haptoglobin is the major hemoglobin-binding protein in the plasma of most vertebrates and all mammals [12]. Haptoglobin is synthesized primarily via hepatocytes

and expressed by a genetic polymorphism as three major genotypes HP1-1, HP2-1, HP2-2 [13]. Both HP 1 and HP 2 alleles have been found in all population, although their frequencies vary considerably among them [14]. The primary physiological function of HP has been described in terms of its interaction with free Hb and prevents hemoglobin deposition in the glomeruli and proximal tubule cells of the kidney and prevents body iron loss [15]. In CKD patients, uremic toxins can shorten the erythrocyte life span leading to hemolysis and contribute to renal anemia [5, 16]. It has been shown that the different HP genotypes have different effects on the rate of uptake and removal of hemoglobin released from erythrocytes [17]. The other possible function of HP is the participation in iron metabolism as HP affects the regulation of the ferroportin (the major transporter of iron) expression and iron transferring from duodenal mucosa to the plasma [18]. The main part of exported iron from duodenal mucosa by the ferroportin is used to sustain de novo erythropoiesis [19]. It has been shown that haptoglobin-null mice export significantly more iron from the duodenal mucosa to the plasma compared to the control counterparts [18]. Few studies have shown the effects of serum HP concentration and its genotypes on body iron status [19, 20] and their association with hemoglobin level is not fully elucidated. We hypothesized that HP can influence on hemoglobin levels, therefore this study aimed to evaluate the probable relationship between hemoglobin levels with HP serum level; its genotypes and inflammation in CKD stage 4 patients with anemia.

Materials and methods

Patients and Study Design

The study was performed in the Department of Biochemistry of Tabriz University of Medical Sciences (TUMS). The ethics committee of TUMS approved the study. Recruitment of the patients occurred from Mar 2011 through Oct 2012. Forty patients (15 males and 25 females) were included in the study. Anemic adult patients (\geq 18 yr) who had CKD stage 4 with GFR = 15 - 29 ml/min/1.73 m² were included in the study. Anemia was defined as hemoglobin levels of less than 13 g/dl for men and postmenopausal women and less than 12 g/dl for premenopausal women [21]. Subjects with transfusion within past 6 months, myocardial infarction history within past 3 months, surgical history within past 3 months, malignancy, diastolic blood pressure greater than 100 mm/hg, uncontrolled diabetes, severe hyperparathyroidism (PTH > 800 pg/ml) and consumers of drugs such as angiotensin receptor blockers and angiotensin-converting enzyme inhibitors were excluded from the study.

Biochemical assays

Blood samples were obtained after an overnight fast and hemoglobin and hematocrit levels were measured by blood cell counter (Sysmex KX-21N, Japane). Separated Serum and whole blood were kept frozen at -80°C until analysis. The serum was analyzed using an auto-analyzer (BT3000, Italy) and manufacture's reagent kits for Mg, hs-CRP, TIBC, Fe, Ca, P, Urea, Cr, Alb and Total protein. Serum HP and ferritin levels were assayed by standard enzyme linked immunosorbent assay (ELISA) kits (Cusabio and DiaPlus, China, USA, respectively). Serum concentrations of IL-6 were measured using human ELISA kits according to the manufacturer's instructions (Bender Med Systems, Vienna, Austria). These biochemical factors were assayed using fully automated ELISA analyzer (TKA Teknolabo Bizet, Italy). The PTH Chemiluminescent Activity Assay kit is designed to measure PTH activity (Liaison, USA) and GFR was calculated by the Cockcroft-Gault formula [22].

Haptoglobin genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the 5-prime ArchivePure DNA blood kit (Ipsogen, Germany). The primer sequences used for amplification of HP1 and HP2 alleles were previously described [23] and listed in Table1. The primers A and B were designed to genotyping the HP alleles via amplification of a 1757-bp length of fragment responsible for HP1 allele and a 3481-bp length of HP2 allele- specific sequence [23]. Detecting the HP2 allele was carried out by amplification of a 349-bp fragment with primer C and D, as well. The 20µL reactions contained 2 U of Taq polymerase (Fermentas Co, Canada), 1–100 ng of DNA, and 200µM dNTPs mix; PCR buffer was used as suggested by the supplier (Fermentas Co, Canada) with no supplements added. After initial denaturation at 95 °C for 5 min, the two-step thermo cycling procedure consisted of denaturation at 94 °C for 45 second and annealing at 62 °C for 1 min (in the presence of primers A, B, C, and D), and extension at 72 °C for 1 min, repeated for 35 cycles, and followed by a final extension at 72 °C for 7 min. The thermocycler instrument used was Veriti 96 well (Applied Biosystems, USA). For genotype assignments, the PCR products were separated in 1% agarose gel (Sigma-Aldrich Co, USA).

The accuracy of genotyping was confirmed by RFLP analysis. The 1757-bp HP1 allele–specific fragment is digested by MlsI enzyme (Fermentas Co, Canada) to two 551- and 1206-bp fragments. Presence of HP2 allele could be detected by digesting the 349-bp amplified product with DraI restriction enzyme (Fermentas Co, Canada) which produced two 193- and 156-bp fragments. After evaluating the accuracy of amplification by two separated primer pairs and PCR-RFLP, they were analyzed by sequencing and blasting them for approaching confidence of genotyping.

Statistical analysis

The collected data were analyzed by statistical methods such as tables and Chi-square, analysis of variance and Pearson's Correlation Coefficient in SPSS software version 16. For all tests, a p value < 0.05 was considered statistically significant.

Results

Clinical features of study subjects were shown in Table 2. Results of PCR genotyping and RFLP for alleles of HP1 and HP2 were displayed in Figure 1. The mean age of all patients was $55.68 \pm$ 14.4 years old. As indicated in table 3, of 40 cases, 15 (37.5%) were male and 25 (62.5%) female. Our results showed that HP2-2 was the most common genotype detected; however patients with HP1-2 were predominantly male. The patients were divided into low (6-9 g/dl) and high hemoglobin levels (9-13 g/dl). The results showed that 9 (22.5%) and 31(77.5%) of subjects had serum low and high hemoglobin levels, respectively. Although, the low levels of hemoglobin were seen in subjects with HP1-2 genotype, but there was no significant relationship between hemoglobin

Table 1. The sequences of the primers used for PCR amplification of HP1 and HP2 alleles

Primer	Sequence	Specific allele	Fragment size
A	'-GAGGGGAGCTTGCCTTTCCATTG-3'	UD1 & UD2	HP1:1757-bp
В	5'-GAGATTTTTGAGCCCTGGCTGGT-3'	$\Pi \Gamma I \alpha \Pi \Gamma Z$	HP2:3481-bp
С	5'-CCTGCCTCGTATTAACTGCACCAT-3'	LID2	240 hr
	5'-CCGAGTGCTCCACATAGCCATGT-3'	ПР2	549-0p

Variable	Mean±SE	Variable	Mean±SE	Variable	Mean±SE
Urea (mg/dl)	126.38 ± 7.12	P (mg/dl)	4.36 ± 0.12	IL-6 (pg/ml)	0.59 ± 3.39
Cr (mg/dl)	3.66 ± 0.15	Hb (g/dl)	10.01 ± 0.24	hs-CRP (mg/l)	10.54 ± 1.89
TIBC (µg/dl)	295.05 ± 8.18	HCT (%)	31.15 ± 0.69	Ferritin (µg/l)	163.83 ± 24.69
Fe (µg/dl)	63.5 ± 4.64	HP (mg/dl)	62.45 ± 9.31	PTH (pg/ml)	111.93 ± 13.35
Ca (mg/dl)	9.03 ± 0.11	Alb (g/dl)	4.21 ± 0.11	GFR (ml/min/1.73 m ²)	21.51 ± 0.74
Mg (mg/dl)	2.01 ± 0.04	Total protein(g/dl)	8.11 ± 0.17	Weight (kg)	72.4 ± 1.73

Table 2. Clinical features of the study subjects

Cr= Creatinine, TIBC= Total Iron Binding Capacity, Fe= Iron, Ca= Calcium, Mg= Magnesium, P= Phosphorus, HCT= He-matocrit, HP=Haptoglobin, Alb=Albumin, IL-6= interleukin-6, PTH= parathyroid hormone, GFR= Glomerular filtration rate

Table 3. Distribution of gender and the level of hemoglobin between the different HP genotypes

Genotype	Total frequency *	Male *	Female *	Hemoglobin level (g/dl)±SE
HP1-1	12.5	20	80	10.54 ± 0.44
HP1-2	15	66.7	33.3	9.35 ± 1.04
HP2-2	72.5	34.5	65.5	10.05 ± 0.26

*The results are noted as percentage

level and HP genotype (p= 0.49). There was no significant relationship between HP genotype and serum HP level (p= 0.32). No meaningful relation was observed between hemoglobin level and serum HP level (p=0.8); however there was a significant positive correlation between hemoglobin level with ferritin and albumin levels. We found a significant negative relationship among hemoglobin level with IL-6 and hs-CRP (p< 0.001 and p< 0.05), respectively. There was a significant positive correlation between serum magnesium and hemoglobin level (p<0.05).

Discussion

CKD is a worldwide public health problem [24]. Anemia is one of the main problems of CKD and occurs especially in the final stages of the disease [25]. It has been shown that the anemia is associated with lower quality of life and a higher risk of adverse outcomes including cardiovascular disease and finally death [26]. HP is one of the plasma proteins that may affect hemoglobin level and the anemia [27]. In the present study we did not find a significant relationship among hemoglobin level with different HP genotypes and serum HP level. In contrast with our findings, some other studies in animal model, thalasemic patients and patients with bone marrow failure syndromes have shown a significant positive relationship between hemoglobin level with HP genotype and serum HP level [19, 28, 29]. In other studies Atkinson et al found a positive significant relationship between HP genotype and hemoglobin level in healthy subjects [30, 31]. This inconsistency may be due to factors such as inflammation, hepatic dysfunction, uremic toxins and renal failure in CKD patients that can change hemoglobin and HP levels [16, 25, 32, 33]. Several studies have indicated that there exists inflammation in CKD patients [32]; it has been shown that inflammation increases plasma HP concentration up to 3- to 8-fold by increased levels of pro-inflammatory cytokines, e.g. IL-6 [34]. Also, inflammatory mediators such as interleukins and tissue necrosis factor, blunt the effect of erythropoietin on the bone marrow, suppress the growth of erythroid colony-forming units (CFU-E), downregulate the expression of erythropoietin receptors on erythroid progenitors and disrupt iron recycling by blocking its release from reticuloendothelial cells [24]. Thus, in CKD patient's chronic and severe inflammation [32] may result in disruption of the balance between hemoglobin and HP levels. Reduction in levels of HP through losing from kidneys, decreased synthesis in liver and reduced erythrocyte life span due to uremic toxins can exacerbate this imbalance [5, 33, 35]. In the best of our knowledge there are no reports on relationship among hemoglobin level with different HP genotypes and serum HP level in CKD stage 4 and it needs further investigation to be elucidated.

We observed significant inverse association among hemoglobin level with hs-CRP and IL-6. The prevalence of inflammation in uremic pati-



Figure 1. Gel electrophoresis for determining haptoglobin genotypes

(A): Genotyping by PCR primers A-B and C-D. Lane 1, 1 Kb DNA ladder; Lane 2, HP1-2 (amplified fragment with 3481 bp length for genotype 2 and 1757 bp length fragment for genotype 2); Lane 3, 349 bp length fragment for showing presence of allele 2, Lane 4, 100 bp DNA ladder. Using primers pair C and D confirmed the accuracy of genotyping by the A and B pair.

(B): Digesting the allele-2 related amplified fragments by DraI for approaching the accuracy of genotyping. Lane 1 & 4, 100 bp DNA ladder. Lane 2, uncut fragment. Lane 3, cut fragments.

(C): Confirming the accuracy of allele-1 related fragment by MlsI digestion. Lane 1, 1Kbp DNA ladder. Lane 2, uncut fragment. Lane 3, cut fragments. Lane 4, 100 bp DNA ladder.

ents is high [36]. Several studies have revealed a strong relationship between anemia and chronic inflammation, and suggest that chronic inflammation is associated with lower hemoglobin concentrations even in the early stages of CKD [36-38]. Our findings are in agreement with the findings of other studies showed that an inverse relationship between hemoglobin level and inflammatory factors such as hs-CRP and IL-6 [39, 40]. Other independent support for this hypothesis is provided by the observation that end stage renal disease patients with elevated blood levels of IL-6 are more anemic, and this anemia is correlated with increased cytokine levels [41]. Evidence suggests several mechanisms by which inflammation may affect the development of anemia [9]. Elevated levels of inflammatory cytokines enhanced oxidative stress and alterations in iron metabolism, conditions associated with inflammatory states, may be implicated in the development of anemia [37, 42]. However, inflammation-induced anemia and resistance to erythropoietin are common features in patients with advanced CKD [24]. In dialysis patients, high CRP levels are associated with low Hb levels and/or erythropoietin resistance [36]. The increased levels of inflammatory cytokines such as IL-6 lead to impaired ability of RBC progenitors to respond to erythropoietin, shortened red blood cell survival and decreased the Hb concentration [7, 36]. Hence, chronic inflammation is an important cause for decreased in Hb level in CKD stage 4.

We also found a significant positive relationship between hemoglobin and ferritin levels which is similar to previous findings [43]. Saigo et al in myelodysplastic syndrome [44] and Locatelli et al in chronic hemodialysis patients [45] showed a significant negative relationship between ferritin and hemoglobin levels, quite the opposite to our findings. These findings contrast with those of Cazzola et al who did not found a significant relationship between hemoglobin and ferritin levels in systemic-onset juvenile chronic arthritis [46]. Availability of iron is key for optimal erythropoiesis and serum ferritin levels in healthy individuals reflect the magnitude of the mobilizable body iron stores, i.e., the iron that is available for the synthesis of hemoglobin in red blood cells [47]. It has been reported that the hemoglobin and ferritin biomarkers were influenced by inflammation [48]. Serum ferritin is an acute phase protein and increases two to fourfold in response to inflammation [36]. Studies in cellular and animal models indicate that inflammatory cytokines such as IL-6 can enhance ferritin synthesis and increase hepatic uptake of serum iron [46]. In turn, increased ferritin expression results in reticuloendothelial iron block and impairs iron absorption [46]. Inflammation leads to blockage in iron utilization and "anemia of chronic disease [49]. Studies have shown that there is a significant association between serum ferritin level and the degree of severity of anemia [50, 51]. More studies are needed to correlate ferritin and hemoglobin level in patients CKD stage 4 with anemia.

In our study there was a significant positive relationship between hemoglobin and albumin levels [41]. Significant positive relationship between hemoglobin and albumin has been shown also in other studies [41, 52]. This finding suggests that anemia is also associated with both cytokine-dependent inflammation and nutritional status of patients.

Conclusion

In conclusion, our study suggests that HP genotype and serum HP levels have no effect on hemoglobin level in CKD stage 4. However, Hb level is associated with inflammation and albumin status. This finding suggests that anemia in these patients is associated with both cytokine-dependent inflammation and nutritional status of patients.

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References

- 1. Hamer, R.A. and A. Nahas, The burden of chronic kidney disease. Bmj, 2006. 332(7541): p. 563-564.
- 2. Hosseinpanah, F., et al., High prevalence of chronic kidney disease in Iran: a large population-based study. BMC Public Health, 2009. 9(1): p. 44.

- 3. Levey, A.S., et al., National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Annals of Internal Medicine, 2003. 139(2): p. 137-147.
- Regidor, D.L., et al., Associations between changes in hemoglobin and administered erythropoiesis-stimulating agent and survival in hemodialysis patients. Journal of the American Society of Nephrology, 2006. 17(4): p. 1181-1191.
- 5. Tsagalis, G., Renal anemia: a nephrologist's view. Hippokratia, 2011. 15(Suppl 1): p. 39.
- Guerrero-Riscos, M.Á., et al., Erythropoietin resistance and survival in non-dialysis patients with stage 4-5 chronic kidney disease and heart disease. Official Publication of the Spanish Society of Nephrology, 2012. 32(3): p. 343-52.
- 7. John, M., et al., Anemia and Inflammation in COPD*. Chest, 2005. 127(3): p. 825-829.
- 8. Perunicic-Pekovic, G., et al., Relationship between inflammatory cytokines and cardiorenal anemia syndrome: Treatment with recombinant human erythropoietin (rhepo). Hippokratia, 2008. 12(3): p. 153.
- 9. de Francisco, A.L.M., P. Stenvinkel, and S. Vaulont, Inflammation and its impact on anaemia in chronic kidney disease: from haemoglobin variability to hyporesponsiveness. NDT plus, 2009. 2(suppl 1): p. i18i26.
- Kanbay, M., et al., Erythropoiesis stimulatory agentresistant anemia in dialysis patients: review of causes and management. Blood purification, 2010. 29(1): p. 1-12.
- 11. Wang, Y., et al., Haptoglobin, an inflammation-inducible plasma protein. Redox report, 2001. 6(6): p. 379-385.
- 12. Wicher, K.B. and E. Fries, Haptoglobin, a hemoglobin-binding plasma protein, is present in bony fish and mammals but not in frog and chicken. Proceedings of the National Academy of Sciences of the United States of America, 2006. 103(11): p. 4168-4173.
- 13. Papp, M., et al., Haptoglobin polymorphism: a novel genetic risk factor for celiac disease development and its clinical manifestations. Clinical chemistry, 2008. 54(4): p. 697-704.
- 14. Carter, K. and M. Worwood, Haptoglobin: a review of the major allele frequencies worldwide and their association with diseases. International journal of laboratory hematology, 2007. 29(2): p. 92-110.
- 15. Gutteridge, J., The antioxidant activity of haptoglobin towards haemoglobin-stimulated lipid peroxidation. Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism, 1987. 917(2): p. 219-223.
- 16. O'Mara, N.B., Anemia in patients with chronic kidney disease. Diabetes Spectrum, 2008. 21(1): p. 12-19.

- 17. McDermid, J. and A. Prentice, Iron and infection: effects of host iron status and the iron-regulatory genes haptoglobin and NRAMP1 (SLC11A1) on hostpathogen interactions in tuberculosis and HIV. Clinical Science, 2006. 110: p. 503-524.
- 18. Marro, S., et al., Lack of haptoglobin affects iron transport across duodenum by modulating ferroportin expression. Gastroenterology, 2007. 133(4): p. 1261-1271.
- 19. Marro, S., et al., Heme controls ferroportin1 (FPN1) transcription involving Bach1, Nrf2 and a MARE/ ARE sequence motif at position– 7007 of the FPN1 promoter. Haematologica, 2010. 95(8): p. 1261-1268.
- 20. Lioupis, C., et al., Association of haptoglobin genotype and common cardiovascular risk factors with the amount of iron in atherosclerotic carotid plaques. Atherosclerosis, 2011.
- 21. Daugirdas, J.T. and T.S. Ing, Handbook of dialysis. 1988: Little, Brown.
- 22. Chudleigh, R.A., et al., Influence of body weight on the performance of glomerular filtration rate estimators in subjects with type 2 diabetes. Diabetes care, 2008. 31(1): p. 47-49.
- 23. Koch, W., et al., Genotyping of the common haptoglobin Hp 1/2 polymorphism based on PCR. Clinical chemistry, 2002. 48(9): p. 1377-1382.
- 24. Hayat, A., D. Haria, and M.O. Salifu, Erythropoietin stimulating agents in the management of anemia of chronic kidney disease. Patient preference and adherence, 2008. 2: p. 195.
- 25. McClellan, W.M., et al., Anemia and renal insufficiency are independent risk factors for death among patients with congestive heart failure admitted to community hospitals: a population-based study. Journal of the American Society of Nephrology, 2002. 13(7): p. 1928-1936.
- Babitt, J.L. and H.Y. Lin, Molecular mechanisms of hepcidin regulation: implications for the anemia of CKD. American journal of kidney diseases, 2010. 55(4): p. 726-741.
- 27. Cox, S.E., et al., Haptoglobin genotype, anaemia and malaria in Gambian children. Tropical Medicine & International Health, 2008. 13(1): p. 76-82.
- 28. Shichishima, T., et al., Low concentration of serum haptoglobin has impact on understanding complex pathophysiology in patients with acquired bone marrow failure syndromes. International journal of hematology, 2010. 91(4): p. 602-610.
- 29. Imrie, H., et al., Haptoglobin levels are associated with haptoglobin genotype and α +-thalassemia in a malaria-endemic area. The American journal of tropical medicine and hygiene, 2006. 74(6): p. 965-971.

- 30. Atkinson, S.H., et al., Seasonal childhood anaemia in West Africa is associated with the haptoglobin 2-2 genotype. PLoS medicine, 2006. 3(5): p. e172.
- 31. Atkinson, S.H., et al., The haptoglobin 2-2 genotype is associated with a reduced incidence of Plasmodium falciparum malaria in children on the coast of Kenya. Clinical infectious diseases, 2007. 44(6): p. 802.
- 32. Mendoza, J.M., et al., Fibroblast Growth Factor 23 and Inflammation in CKD. Clinical Journal of the American Society of Nephrology, 2012.
- *33. Fabrizi, F., et al., Hepatic disorders in chronic kidney disease. Nature Reviews Nephrology, 2010. 6(7): p. 395-403.*
- Dobryszycka, W., Biological functions of haptoglobin-new pieces to an old puzzle. European journal of clinical chemistry and clinical biochemistry, 1997. 35(9): p. 647-654.
- 35. Jamall, S., et al., Distribution of plasma proteins in patients with chronic renal disease and hepatic insufficiency. Pakistan Journal of Biochemistry and Molecular Biology (PJBMB), 2011. 44(1).
- Bárány, P., J.C. Divino Filho, and J. Bergström, High C-reactive protein is a strong predictor of resistance to erythropoietin in hemodialysis patients. American journal of kidney diseases, 1997. 29(4): p. 565-568.
- Chonchol, M., et al., Association of inflammation with anaemia in patients with chronic kidney disease not requiring chronic dialysis. Nephrology Dialysis Transplantation, 2008. 23(9): p. 2879-2883.
- 38. Siribamrungwong, M. and K. Puangpanngam, Treatment of Periodontal Diseases Reduces Chronic Systemic Inflammation in Maintenance Hemodialysis Patients. Renal Failure, 2012(00): p. 1-5.
- 39. Van Lettow, M., et al., Low plasma selenium concentrations, high plasma human immunodeficiency virus load and high interleukin-6 concentrations are risk factors associated with anemia in adults presenting with pulmonary tuberculosis in Zomba district, Malawi. European journal of clinical nutrition, 2005. 59(4): p. 526-532.
- 40. Poveda, G.F., et al. Pattern of blood levels of erythropoietin and proinflammatory cytokines in patients with anemia of chronic disorders secondary to infection]. 2001.
- 41. Owen, W.F., C-reactive protein as an outcome predictor for maintenance hemodialysis patients. Kidney international, 1998. 54(2): p. 627-636.
- 42. Nangaku, M. and K.U. Eckardt. Pathogenesis of renal anemia. 2006. Elsevier.
- 43. Fievet, P., et al., Treatment of iron deficiency in predialysis state by low molecular weight iron dextran high doses intravenously. Nephrologie & therapeutique, 2011.

- Saigo, K., et al., Oxidative Stress Levels in Myelodysplastic Syndrome Patients: their Relationship to Serum Ferritin and Haemoglobin Values. The Journal of International Medical Research, 2011. 39(5): p. 1941-1945.
- 45. Locatelli, F., et al., Predictors of haemoglobin levels and resistance to erythropoiesis-stimulating agents in patients treated with low-flux haemodialysis, haemofiltration and haemodiafiltration: results of a multicentre randomized and controlled trial. Nephrology Dialysis Transplantation, 2012.
- Cazzola, M., et al., Defective iron supply for erythropoiesis and adequate endogenous erythropoietin production in the anemia associated with systemic-onset juvenile chronic arthritis. Blood, 1996. 87(11): p. 4824-4830.
- 47. Milman, N. and M. Kirchhoff, Relationship between serum ferritin, alcohol intake, and social status in 2235 Danish men and women. Annals of hematology, 1996. 72(3): p. 145-151.
- 48. Mburu, A.S.W., et al., The influence and benefits of controlling for inflammation on plasma ferritin and hemoglobin responses following a multi-micronutrient supplement in apparently healthy, HIV+ Kenyan adults. The Journal of nutrition, 2008. 138(3): p. 613-619.
- 49. Hsu, C., C.E. McCulloch, and G.C. Curhan, Iron status and hemoglobin level in chronic renal insufficiency. Journal of the American Society of Nephrology, 2002. 13(11): p. 2783-2786.
- 50. Gunnell, J., et al., Acute-phase response predicts erythropoietin resistance in hemodialysis and peritoneal dialysis patients. American journal of kidney diseases, 1999. 33(1): p. 63-72.
- 51. Kalantar-Zadeh, K., R.A. Rodriguez, and M.H. Humphreys, Association between serum ferritin and measures of inflammation, nutrition and iron in haemodialysis patients. Nephrology Dialysis Transplantation, 2004. 19(1): p. 141-149.
- 52. Abraham, G., et al., C-Reactive protein, a valuable predictive marker in chronic kidney disease. Saudi Journal of Kidney Diseases and Transplantation, 2009. 20(5): p. 8-11.

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History of fatty liver in medieval Iranian medicine

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Abstract

Today's society is especially vulnerable to fatty liver disease (FLD) due to its high prevalence and caused by high level of triglyceride accumulation irreversible complications as well as absence of non in hepatocytes (2). Fat accumulation in liver cells invasive diagnostic methods. There is insufficient can eventually lead to inflammation and more proknowledge of pathogenesis of fatty liver and also blems in these cells, and if left untreated, it can there are several causes which develop this disease, progress to cirrhosis (1-3). This disease is cauin other words it is a sort of multifactorial disease. sed by various factors such as alcohol abuse and Thus, no effective and proven treatment has been obesity (4) and is classified into two categories of introduced for it and only reducing major risk fac- alcoholic and nonalcoholic fatty liver (5). Notors such as obesity and hyperlipidemia as well as nalcoholic fatty liver disease (NAFLD) affects 20controlling of diabetes are emphasized. There is a 35% of individuals in industrialized nations (1,2,4) worldwide positive approach to Complementary Further, NAFLD occurs in about 2.6% of children and Alternative Medicine (CAM) and World Health and up to 53% of obese children are diagnosed Organization (WHO) emphasizes on using existing with NAFLD and is the most prevalent condition methods offered by CAM. In this study Avicenna's among chronic liver diseases (1). Recent Studies opinions - (980-1037A.D) physician and noble showed an increase in prevalence of NAFLD in scholar of Iranian Traditional Medicine- on fatty li- Iran and latest estimates suggests a prevalence of ver are described. This study resulted in that fatty 30%(5). Also this disease is the most prevalent liver is caused by coldness and moisture of liver silent disease in Tehran's population as a modern and increased phlegm level in body as well. Due to developing capital (9). In 40-90% of cases, obethe cause various diagnostic, preventive and thera-sity leads to NAFLD (4) and 10-20% of individupeutic methods are introduced. Some of these the- als affected by NAFLD will develop nonalcoholic rapeutic methods such as weight loss and exercise steatohepatitis (NASH) (5). Possibility of NASH are confirmed by modern medicine so far. There are progression to cirrhosis is approximately 3-15% other recommendations for therapeutic procedure (5) and cirrhosis eventually can lead to hepatocelwhich need more investigations. Studies on causes lular carcinoma (HCC) (1,3,6). Most patients who of FLD and its symptoms based on Avicenna's view have fatty liver show no clinical signs. In most cacould open the new way to control and treat FLD ses fatty liver has no obvious symptoms (7) thereand reduce burden of this highly prevalent disease fore many of those affected by this disease has no in society subsequently.

phlegm, cold intemperament of the liver, Avicenna, several causes which develop this disease (8), in Iranian traditional medicine.

Introduction

Fatty liver is a relatively prevalent disease that awareness of it. There is insufficient knowledge of Keywords: Fatty liver, temperament, humor, pathogenesis of fatty liver (2) and also there are other words it is a sort of multifactorial disease. Thus, no effective and proven treatment has been introduced for it and only reducing major risk factors such as obesity and hyperlipidemia as well as controlling of diabetes are emphasized (10). High prevalence of fatty liver, absence of specific clinical signs and scarcity of safe and exact clinical methods of diagnosis and screening for this disease along with its dangerous complications has made it a matter of increasing concern (7). Any initiative which proves itself effective in controlling of this silent disease is valuable in promoting society's health through reducing burden of this disease.

WHO's Approach to Complementary and Alternative Medicine

The use of traditional medicine and herbs, as one of the WHO's goals, was proclaimed in "Global Strategy for Health for All by the Year 2000". Subsequently, WHO recommended the use of traditional medicine and herbs in policies and administrative programs of Member States as a part of their national drug policies and introduced guidelines and procedures for achieving this goal (11).

According to WHO's definition, traditional medicine is the sum total of skills and practices that were used to restore and maintain health before the evolution of modern medicine. Traditional medicine is defined as "health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-being"(11).

In developed countries there is an emerging trend to CAM among ordinary people. They believe alternative medicine is not as invasive and harmful or toxic as modern therapeutic methods. In Japan, Australia, Canada and Switzerland nearly 40 to 70 percent of people have used complementary medicine and this has induced many of modern physicians to deploy some form of CAM in treatment their patients (12).

Consequently, WHO recognizes an important role for traditional medicine in Developing Countries and admits useful assistance of traditional systems in providing health care services for large numbers of people. Due to easy access to local herbs and other traditional therapeutic methods, traditional medicine treatments are at low costs. Therefore WHO supports regional governments in increasing research activities and educational facilities connected with traditional medicine and encourages them to do so (13). With regard to worldwide positive approach to CAM *and* WHO's recommendations on the necessity of consideration and promotion of them, in this study Iranian Traditional Medicine (ITM) views on fatty liver based on Avicenna's opinions are explained.

Iranian Traditional Medicine (ITM) as an independent system of Medicine

Nowadays the world is moving to integrating complementary and alternative medicine to the classic medicine for decreasing side effects and costs and increasing efficacy. The Iranian traditional medicine (as has been known Unani Medicine) is one of the ancient schools of traditional medicine.(14)

The most important Persian physicians were Abu Bakr Muhammad Ibn Zakariya Al-Râzi , Ali Ibn Al-Abbas-al-Ahvazi (Haly Abbas; 949–82), and Abu Ali Al-Hussain Ibn Abdullah Ibn-e-Sina. The texts Qanoon fel teb (The Canon) by ibn-e-Sina, Râzi's Kitab al-hawi (Continens), and Kitab-al-Maliki (Liber Regius) by Haly- Abbas were basic and cardinal references for western medical science from the 13th to the 18th centuries.(15)

Ibn Sina by his Latinized name Avicenna (980-1037) was a headmost Iranian physician, theologian and polymath who wrote almost 450 treatises on a wide range of subjects, of which around 240 have survived. In particular, 150 of his surviving treatises concentrate on philosophy and 40 of them concentrate on medicine. His most famous works are The Book of Healing (shefa), a vast philosophical and scientific encyclopedia, and The Canon of Medicine, which was a standard medical text at many medieval universities. The Canon of Medicine was used as a training guide for scholars in the Europe and Islamic lands for nearly a millennium. Ibn Sina is considered as the father of modern medicine and clinical pharmacology. In addition, he is known to have been a major impact on both medicine and the development of logic and Philosophy in medieval Europe.(16)

To understand of Iranian traditional medicine (as has been known Unani Medicine), it is necessary to explain some principles. (14)

ITM is a perfect and dynamic scientific school with tested principles which regards the universe as the work of an all-knowing, all-wise Creator.

Iranian traditional medicine system makes an attempt to provide the best possible way that a person can lead a healthy and good lifestyle with minimal disease. In ITM, disease prevention is considered more important than treatment and body's physiological functions are consider on basis of seven natural components called Umoor-e-Tabaiyah (Doctrine of Naturals). Umoore-Tabaiyah consists of Arkan (Elements), Mizaj (Temperament), Akhlat (Humors), Aaza (Organs), Arwah (Spirits), Quwa (Faculties), Afaal (Functions) (14).

From the viewpoint of Iranian traditional medicine, the body is composed of the four humors: Blood (Dam), Phlegm (Balgham), Yellow bile (safra), and Black bile (Sauda). (16)

"Humor" is a wet and fluid Material which foodstuffs in the first stage of permutation changes to it. It is called in the Iranian traditional medicine texts as "Khilt". (14)

Any humor in the temperament and its activity is similar to one of the four elements (Air, Water, Fire and Earth). (16) Each of the humors was related with pairs of qualities; So that Quality or disposition of yellow bile is hot and dry, blood is hot and wet, phlegm is cold and wet, black bile is cold and dry.(14, 17) Each of the four humors have certain and necessary duties that in case their normal quality and quantity, human health are provided. (18) More or less the amount of humor, are considered abnormal. Therefore the foundation of health is the right ratio and specific balance of humors based on their quality and quantity. (16) Liver with the production of these humors, play essential role in human physiological live.

Any humors divided in two categories:

-Normal humor

-Abnormal humor

Normal humor causes health and abnormal causes Diseases (17) According to approach in this article that explains association between fatty liver and phlegm and the better understand this humor, we will describe briefly normal and abnormal properties of phlegm. The phlegm is similar to water element, therefore, this humor has some different characteristics like flexibility or ease plasticity and softness. (16) Normal phlegm with passing specific processes is produced in the liver with other

humors and then it flow to blood in the body in addition to provide appropriate food for absorption by the total body, provides feeding phlegmatic organs including brain and spinal cord. Normal phlegm also considered as a store for food of organs. For example for any reason when the body becomes deficient in the blood, phlegm convert to the blood and need to make it go away; on the other hand if the nutritional needs of body tissues do not provide, this store will act. (18)

The first material for the production of normal phlegm in the body is wet and cold foods. Some of the most important of these foods include fish, milk, yogurt and etc. Other Factors that affect the production of phlegm in the body are normal digestion in the stomach, adequate sleep, suitable rest, relaxation and etc. Also coldness of temperament liver leads to more phlegm production in the body. (18) On the other hand abnormal phlegm are produced in conditions of excess intake in the phlegm-producing foods such as thick and sticky foods (like head and leg, pasta, olovie salad), cold and wet foods and other conditions such as excessive sleeping, lack of exercise and abnormal gastrointestinal tract. (18)

Avicenna's Viewpoints on Fatty Liver

In ITM, liver, brain and heart are three principal organs which are called *Aza-e Raeesa* (Principal Organs) for their important roles in maintaining life (18). According to ITM, health is attributed to the equilibrium of four Akhlat (Humors) in the body while disease occurs due to disturbances in the equilibrium of akhlat (17). Since all four of these humors are generated by *liver (18), this organ has a vital role in maintaining health. In ITM manuscripts, fatty liver has not been cited under this title but there are detailed examinations of physiopathology*, etiology, symptoms and treatment of this disease under the title of coldness and moisture of liver which will be explained in this study.

Physiopathology

As was described, when there is a change in quantity or quality of humors, the equilibrium of them is disturbed and the body deviates from health state (17). Fatty liver is caused by coldness and moisture of liver and elevated phlegm. A cold intemperament of the liver causes deposit and accumulation of blood lipids in liver (17).

Avicenna has described elevated levels of lipids in blood as "Dosoomat Al-Dam". "Dosoomat" means "fatty, oily" and "Dam" means "blood". According to ITM findings, the blood circulating in vessels is a combination of four Humors (Akhlat) in which blood lipids can be a part of these humors (19). Ali ibn al-'Abbas Ahvazi says that blood lipids in warm bodies is used to generate heat but in cold bodies it maintains in blood and is brought to organs through vessels. It dissolves in organs with a warm temperament while in organs with cold temperament it solidifies into fat (Shahm) (17). Due to this reason there is an excess of fat in Amaa (intestines & viscera) while there are fewer amounts of fat in liver (18).

Therefore, if a cold intemperament occurs in liver, for any reason, there would be an accumulation of fat causing fatty liver. On the other side, more amounts of phlegm are produced in cold and moist temperaments (18). Therefore, practitioners of ITM believe, persons who congenitally have a cold and moist temperament in their body are more susceptible to increased fat production in their body and development of fatty liver. This issue confirms the role of genetic factors in causing the disease, as modern medicine has certified it (1).

Etiology

Increased phlegm and coldness of liver, as main causes of fatty liver, are attributed to several factors. Following is a review of them.

Production of normal khilt is dependent on proper digestion of food in stomach therefore stomach weakness and impairments in process of nutrients digestion leads to increased phlegm production in liver (14). Eating fast, less chewing and *irregular meal times cause impairments in process of food digestion in stomach and lead to phlegm production (20).*

In cases when there is no impairment in stomach function, excessive intake of phlegm-producing foods increases phlegm production in liver (18). When a cold intemperament occurs in liver and is dominated by moisture, phlegm production increases. One of the most important factors for coldness of liver is coldness of body (21), so that an increase in coldness and moisture of body causes coldness and moisture of liver which resulted in increasing phlegm production (17). Sedentary lifestyle, overeating, oversleeping or hypersomnia (especially after-lunch napping) are factors which increase coldness and moisture of the body and phlegm production in liver (22). Drinking cold water in fasting state, after bathing or sexual intercourse is another cause of coldness of liver (23).

In ITM, the relation between fatty liver and obesity is explained by referring to increased phlegm production as cause of obesity (17). All factors that are mentioned above for increasing phlegm in the body can develop obesity. An increase in coldness and moisture of the body of obese persons caused by increased intake of phlegm producing foods, stomach disorders or increased coldness and moisture of liver can lead to more phlegm production and consequently can progress fat accumulation and fatty liver (23).

Symptoms

General symptoms of increased phlegm in the body include fatigue, weakness and asthenia of body, emesis, anorexia (24) which are also affirmed in modern medicine as symptoms of FLD in marked patients (5).

The other symptoms of increased phlegm or coldness of liver can follow dyspepsia (25), sour belch or heartburn (23, 25), sour taste in mouth (23), abnormal bowl movements (constipation or diarrhea) (23), decreased thirst, white lips and tongue (23), ill-colored face (23, 25), white, thick and phlegmatic urine (18), weak pulse (23, 25), swollen eyes and face (23, 25), excessive saliva or sialorrhea, excessive sleeping (17, 22), reduced perceptivity (17, 22).

As explained in etiology, weak function of stomach develops impairment in digestion and increases phlegm in the body there after. Thus, the existence of some of digestive symptoms can be considered as *prognostic factors* for developing fatty liver. However, further clinical studies and evidences are needed.

Prevention and Treatment

Prevention

ITM has dedicated more regarding to the prevention of disease rather than its cure. Maintaining health have very worth so that the main work of the physicians were to keep people healthy and to treat them if they became sick (11). In Avicenna's teachings there are specific recommendations for maintaining health and preventing diseases under title of Hifz-e-seha (Normal life style) (26). There are six agents which are necessary for the maintenance of good health, which is called as: Setteh-e-Zarurieah. These essential factors consist of : Air, Food and Drink, Sleep and Wakefulness, Evacuation and Retention, Body movement and Repose, Mental movement and Repose (11).

Many diseases are prevented by adherence to them. These tips are cited for any organs of the body in different temperaments and age groups accordingly in separate chapters. The following are instructions which could be useful for maintaining liver health and preventing fatty liver progression. These include, avoidance of overeating, avoidance of constant intake of phlegm-producing foods, avoidance of drinking cold water with meals, in fasting state, after bathing and sexual intercourse, observing proper intervals between meals, eating slowly and chewing much more, *proper body exercise, avoidance of oversleeping specially after-lunch or day time napping (23).*

Treatment

Based on ITM the onset of disease condition refer to the imbalance in body temperament and humors. So that, treatment is based on the correction of temperament and humors to receive a balanced state. Because every humor has a specific temperament So, the drug used for the treatment should have been the opposite temperament than that of the diseased humor, resulting in normalization of the temperament. A drug which has hot temperaments, can treat a diseases, which is cold in nature and so on (11).

ITM physicians have given four Method of treatment:

- 1. Hifz-e-seha (Correction of the six essentials)
- 2. Tadbir ba Ghaza (Diet therapy)
- 3. Tadbir ba Dawa (Pharmacotherapy)

4. Aamale yadaavi (Manipulation or physical therapy)

These modes might also be considered as levels of treatment, where if a level fails or is inadequate

the next level is considered (11). Avicenna believed that curing patient in general is prior to curing disease and different patients with the same disease may have different temperaments. He insisted that these points should be considered in adoption and implementation of treatment (24). Treatments implemented for all patients include, nutritional therapies, drug therapies, manual therapies such as massage therapy, cupping, *Phlebotomy*, etc. respectively (26). Observing above considerations, some of most important treatments for fatty liver are reviewed.

The best food for patients affected by fatty liver is chickpea stew with meat of partridge, francolin or See-see Partridge (Ammoperdix griseogularis) and also chick with cinnamon (24). Medication include single drugs and compound drugs. Included in single drugs are Foeniculum vulgare Mill, Solanum nigrum, celery seed (Apium graveolens seed) and liquorice root (Glycyrrhiza glabra L.). Moreover, there are various compound drugs such as Itrifal-e-Kabir, Dawa-ul-Kurkum, Maajun Falasfa, etc, which detailed instructions to make and prescribe them are mentioned in respective sources. In addition, Avicenna emphasized body exercise and dietary regimen (Increasing physical activity and a balanced food intake reduction) and they have warned against excessive weight reduction (24).

Conclusion

Regarding high prevalence of FLD and its *irre-versible complications* as well as the absence of effective diagnostic, preventive and therapeutic methods for this disease, viewpoints relating to FLD in ITM and also Avicenna's opinions in this field were reviewed in this study and Any initiative which proves itself effective in controlling of this silent disease is valuable in promoting society's health through reducing burden of this disease.

WHO has admitted the important role of traditional medicine in Member States and has emphasized the necessity of effective use of it. So it is an important professional task to search for non invasive, effective and safe diagnostic and therapeutic methods for FLD. The school of ITM is one of the oldest schools of medicine in the world. It is based on the doctrine of the four Humors (Akhlat). ITM sees health as equilibrium of the body determined by quantities and qualities of the four humors. Liver as an organ which produces humors and as one of the principal organs in the body (Aza-e Raeesa) has been in focus of Hakims' attention. In this regard, anatomy, physiology, pathology and semiology of liver as well as preventive, therapeutic and prognostic methods for liver diseases are explained under respective headings. According to Avicenna's teachings, increased phlegm production or cold intemperament of the liver, if caused by any reason, develops fat accumulation in liver. Causes of increased phlegm are stomach weakness, impairments in nutrients digestion and increased intake of phlegm-producing foods. On the other side, coldness and moisture of body causes coldness and moisture of liver and increased phlegm production. Sedentary lifestyle, overeating, oversleeping or hypersomnia (especially after-lunch napping) are among other factors which increase coldness and moisture of the body and consequently increase coldness and moisture of liver and phlegm production in liver. Drinking cold water in fasting state, after bathing or sexual intercourse is another cause of coldness of liver.

Also obesity as one of the major causes developing fatty liver is interacting with coldness and moisture of liver and increased phlegm. Avicenna's therapeutic viewpoint on this case includes preventive recommendations and therapeutic methods including avoidance of factors developing increased phlegm or coldness and moisture of liver such as overeating or oversleeping, intake of compound drugs such as Itrifal-e-Kabir, Dawa-ul-Kurkum, Maajun Falasfa and herbs such as fennel, black nightshade, celery seed and liquorice root. Additionally, recommendations for health maintenance are provided under title of Hifze-seha. Since causes and symptoms of this disease are described on basis of Avicenna's teachings, it is helpful to prepare the ground for clinical researches utilizing ITM's guidelines in this field to reduce the burden of this disease in society as possible.

References

- Wei Y, Rector RS, Thyfault JP, Ibdah JA. Non-alcoholic fatty liver disease and mitochondrial dysfunction. World J Gastroenterol 2008, 14(2):193-199. doi: 10.3748/wjg.14.193
- 2. Basaranoglu M, Kayacetin S, Yilmaz N, Kayacetin E, Tarcin O, Sonsuz A. Understanding mechanisms of the pathogenesis of nonalcoholic fatty liver disease. World J.Gastroenterol., 2010, 16(18), 2223-2226. doi: 10.3748/wjg.v16.i18.2223
- Assy N., Nassar F., Nasser G., Grosovski M. Olive oil consumption and non-alcoholic fatty liver disease. World J Gastroenterol 2009 April 21; 15(15): 1809-1815. doi: 10.3748/wjg.15.1809
- Takahashi Y, Toshio Fukusato. Pediatric nonalcoholic fatty liver disease Overview with emphasis on histology. World J Gastroenterol 2010 November 14; 16(42): 5280-5285. doi: 10.3748/wjg.v16.i42.5280 PMCID: PMC2980676
- 5. Abolhasani M, Mohammadi G, Karbaschian Z. nutrition and fatty liver. 1 ed. Alavian, editor. tehran: teimorzade, tabib; 2012.
- Rocha R, Cotrim HP, Bitencourt A, Barbosa D, Santos A, Almeida A, Cunha B, Guimarães I. Nonalcoholic fatty liver disease in asymptomatic Brazilian adolescents. World J Gastroenterol 2009 January 28; 15(4): 473-477 doi: 10.3748/wjg.15.473 PMCID: PMC2653370
- Schreuder TC, Verwer BJ, Nieuwkerk C, Mulder C. Nonalcoholic fatty liver disease: An overview of current insights in pathogenesis, diagnosis and treatment. World J Gastroenterol. 2008 April 28; 14(16): 2474–2486. doi: 10.3748/wjg.14.2474 PMCID: PMC2708357
- 8. Chiang PH, Chang TY, Chen JD. Synergistic effect of fatty liver and smoking on metabolic syndrome. World J Gastroenterol 2009 November 14; 15(42): 5334-5339. doi: 10.3748/wjg.15.5334 PMCID: PMC2776862
- Sotoudehmanesh R, Sotoudeh M, Ali-Asgari A, Abedi-Ardakani B, Tavangar SM, Khakinejad A, Sadeghi Z, Malekzadeh R. Silent liver diseases in autopsies from forensic medicine of Tehran. Arch Iran Med. 2006;9:324–328. [PubMed]
- Al-Gharabally A, O'Brien CB, Acosta RC. A Pilot Study of Pioglitazone for the Treatment of Non-Alcoholic Fatty Liver Disease. Hepatitis Monthly. 2007;7(3):131-7.
- 11. Rezaeizadeh H, Alizadeh M, Naseri M, Ardakani MRS. The traditional Iranian medicine point of view on health and disease. Iranian Journal of Public Health. 2009;38(Suppl. 1).

- 12. Mohsen N. Introduction to Iranian traditional medicine. In: Group ITMR, editor. Introduction to Iranian traditional medicine. Tehran: Tehran University of Medical Sciences; 2006. p. 31-27, 76-34, 120-79.
- 13. Organization WH. WHO traditional medicine strategy 2002-2005. World Health Organization Geneva; 2002.
- 14. Emtiazy M, Keshavarz M, Khodadoost M, Kamalinejad M, Gooshahgir S, Bajestani HS, et al. Relation between Body Humors and Hypercholesterolemia: An Iranian Traditional Medicine Perspective Based on the Teaching of Avicenna. Iranian Red Crescent Medical Journal. 2012;14(3):133-8. PMID: 22737569 PMCID: PMC3372029
- 15. Gorji A, Ghadiri MK. History of headache in medieval Persian medicine. The Lancet Neurology. 2002;1(8):510-5.
- 16. Choopani R, Mosaddegh M, Gir A, Emtiazy M. Avicenna (Ibn Sina) aspect of atherosclerosis. International journal of cardiology. 2012. PMID: 22357428
- 17. Ahvazi AiA. kamel alsanaat altebbie. In: Medicine RIoN, editor. kamel alsanaat altebbie tehran: Jalal aldin; 2008. p. 118-28.
- 18. Ibn Sina A. Al-Qanun fi al-Tibb. In: IS a-D, editor. Medicine Lebanon: Alamy Le- Al-Matbooat institute; 2005. p. 41-52.
- 19. Emtiazi M, Nazem E, Keshavarz M, Kamalinejad M, Goosshehgir S, Hashem Dbbaghian F, et al. Avicenna Medicine and Hyperlipidemia. Jornal of Isslamic and Iranian Traditional Medicie. 2011.
- 20. Nasseri M ea. General overview of the traditional medicine of Iran. tehran: published in cooperation with the Institute of Iranian Traditional Medicine Publications; 20011.
- 21. Ahvazi AiA. kamel alsanaat altebbie. In: Medicine RIoN, editor. kamel alsanaat altebbie tehran: Jalal aldin; 2008. p. 83-5.
- 22. Ibn Sina A. Al-Qanun fi al-Tibb. In: IS a-D, editor. Medicine. Lebanon: Alamy Le- Al-Matbooat institute; 2005. p. 158,73.
- 23. Ibn Sina A. Al-Qanun fi al-Tibb. In: IS a-D, editor. Medicine. Lebanon: Alamy Le- Al-Matbooat institute; 2005. p. 131-3.
- 24. Aqili Khorasani S. Moalejate Aghili. tehran: Tehran university of Medical Science: Institute for Islamic and Complementary Medicine; 2008.
- 25. Ahvazi AiA. kamel alsanaat altebbie. In: Medicine RIoN, editor. kamel al-sanaat al-tibbie tehran: Jalal aldin; 2008. p. 529.
- 26. Ibn Sina A. Al-Qanun fi al-Tibb. In: IS a-D, editor. Medicine. Lebanon: Alamy Le- Al-Matbooat institute; 2005. p. 215, 69.

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Hopelessness and depression among college students in Sivrihisar in Eskisehir: An epidemiological study from Turkey

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Abstract

Aim: This study aimed to evaluate the level of hopelessness and depression among college students.

Method: This Study is a descriptive study, it was realized between from 05 September 2011 to 25 October 2011 among college students of Sivrihisar in Eskisehir from Turkey. And 1101 (79.0%) students participated to this study. The survey forms were filled by students under supervision. Beck Hopelessness Scale was used for evaluation of hopelessness, Beck Depression Scale was used for evaluation of depression. One-way ANOVA, Student's t-test and Spearman correlation analysis were used for statistical analysis, p <0.05 was accepted for statistical significance.

Results: Of this study group 521 (47.3%) were men. The average age was 15.77 ± 1.20 (min: 14, max:20) years. In this study, prevalence depression was 28.4% (n=313). The average point of students from Beck Hopelessness Scale was 5.22 ± 3.99 (min: 0, max: 19). A significant positive relationship between Beck Hopelessness Scale points and Beck Depression Scale points among students was determined (p<0.001).

Conclusion: In this study, a significant positive relationship between depression and hopelessness were determined.

Keywords: Hopelessness, depression, college students.

Introduction

Mental disorders in the adolescent period are one of the major causes of morbidity and mortality. The hopelessness is one of these mental disorders. It has been defined as negative expectations about the future by Beck et al. (1) and as an important factor in understanding the depression from a cognitive perspective as well as a motivational/cognitive condition that is characterized by negative expectations for the future by Velting (2). On the other hand, Dilbaz and Seber (3) suggested that hopelessness is a mood disorder that adversely affects the mental health of the individual as well as causes psychological problems such as depression and suicide and also constitutes a part of these clinical conditions. The education and training problems in adolescence, in which period the psychosocial development is turbulent, and as well as other factors such as unemployment, deprivation, and poverty and negative experiences in childhood are well-known factors that increase the level of hopelessness in adolescents (4, 5, 6).

The hopelessness is a common health problem particularly among adolescents and it can be a symptom of or a risk factor for depression. In a study of college students in Turkey, 17.4% of the students have been reported to be hopeless about the future (7). The depression and hopelessness has been suggested to be risk factors for the different types of suicidal behavior including completed suicide, suicide attempts and suicidal ideation (8, 9, 10, 11). Moreover, Kashani and colleagues (12) suggested that a high level of hopelessness not only increases depression and suicide but also increases risk of all kinds of psychopathology. Hopelessness also increases the morbidity of some diseases such as myocardial infarction and cancer, and is an important factor known to negatively affect the quality of life (13). Depression manifests as a mental disorder such as intense sorrow or grief, insomnia, decreased appetite, anhedonia, hopelessness, self-dislike, and suicidal ideation (14). There are some researchers reporting a strong positive correlation between hopelessness and depression (15, 16). This study aimed to evaluate the level of depression and hopelessness among collage in Sivrihisar in Eskişehir from Turkey.

Material and methods

This is a descriptive study that was conducted from September 5 to October 25, 2011 on students who have been studying in the college in the town center of Sivrihisar in Eskischir from Turkey. Sivrihisar, which is located to the east to Eskischir, is a town 100 km away from the Eskischir city center. According to the results of Turkey Statistical Institute 2010 address-based population registration system, the total population in the town is 23488, with 11579 (49.3%) are men and 11909 (50.7%) are women. Approximately 30% of the population was living in the town center, 15% was living in centre and 55% was living in villages or uplands. The people are mainly engaged in agriculture and animal husbandry.

There are a total of 8 colleges providing training and educational services in the town center. According to the records of the Directorate of National Education, a total of 1394 students were studying in these schools: 130 in Sivrihisar Vocational School of Health, 93 in Sidika Hanim College, 76 in Imam Hatip College, 159 in SEV Anatolian College, 177 in Zubeyde Hanim Girls' Vocational College, 130 in Anatolian Vocational College, 219 in Fahri Keskin Anatolian Teacher Training College and 410 in Technical and Industrial Vocational College. Of them, 1101 students (79.0%) constituted the study group.

A questionnaire form was prepared in the light of relevant literature (6, 17, 18, 19). The survey form consists of three parts and the first part contains questions about the students' sociodemographic characteristics (age, sex, school, class, residence and the presence of his/her own room, family income level, family type, personality type, and number of siblings) and some factors that are thought to be associated with hopelessness (such as chronic disease history, presence of physical disabilities, previous history of mental illness, depression, history of prior adverse event affecting the life, the presence of acne vulgaris, smoking status, overweight / obesity, the presence of the mother / father, and parental education level and employment status). The second and third parts of the questionnaire include questions related to the Beck Hopelessness Scale (BHS) and Beck Depression Inventory (BDI), respectively.

Before the collection of data, requisite consents were obtained from Sivrihisar Town National Education Directorate and the time of the interview was determined. The colleges were visited and the students were gathered in a classroom. All students were informed about the aim of the study. After obtaining informed verbal consent, the questionnaire forms were given to each student and they completed the form within 20-25 minutes under supervision. A total of 293 (26.6%) students, including 283 students who were not present at the school at the time of the interview and 10 students who gave false/incomplete information were excluded from the study.

The BHS was used in this study for the evaluation of the level of hopelessness. This scale has been developed by Beck et al. in 1974 (20) and the validity and reliability of the scale in Turkey were tested by Seber and colleagues (21). The BHS is a self-assessment tool consisting of 20 questions with "yes" and "no" options. The possible total score is between 0 and 20, and the level of hopelessness increases with the increasing total score.

The BDI was used for the assessment of depression in our study. The scale has been developed in 1961 by Beck et al. (22). The validity and reliability of the scale in Turkey were tested by Hisli (23). The BDI is a self-assessment scale that comprises 21 questions, answered on a 4-point Likert-like scale and the questions are answered in consideration of the last 15 days. Each answer was scored as 0, 1, 2 or 3. Total score ranges from 0 to 63, and the cut-off score is 17. The students smoking at least 1 cigarette per day were considered as "smoker" (24). The students who defined herself/himself as hectic-enthusiastic-hasty-impatient were considered as having "type A" personality and those defining as quiet-quiet-define patient-planned-programmed were considered as having "type B" personality (25). Following the completion of questionnaire, the students' body height was measured with a tape-measure and body weight was measured with a home-type scale. The students with a Body Mass Index (BMI) of 25 and over were defined as overweight and obese (26). The presence of acne vulgaris was evaluated by physical examination. The Statistical Package for Social Sciences (SPSS) version 15.0 (Chicago, IL) was used to enter and analyze the data on

a personal computer. Data were evaluated through student t test, One way Anova and Spearman correlation analysis. The measure for statistical significance was established a priority as p < 0.05.

Results

The study group consisted of 580 female (52.7%) and 521 male (47.3%) students. The

mean age was 15.77 ± 1.20 years (range, 14-20 years). Of the students, 476 (43.2%) were aged 15 years or below, 540 (49.0%) were between the ages of 16-17 years, and 85 (7.7%) were aged 18 years or above. The mean score on BHS was 5.22 \pm 3.99, ranging from 0 to 19. The distribution of mean BHS scores according to some characteristics of the students is given in Table 1. The frequency of depression was found to be 28.4% (n =

Table 1. Distribution of mean BHS scores according to some characteristics of the students

		DUC Caoro	Statistical
		DITS Score	analyses
Some characteristics	n	X±Sd	t/F: n
Gende			
Girl	580	4.27±3.61	
Boy	521	6 28±4 13	8 644: 0 001
A ge group	(vear)	0.20-1.10	0.044, 0.001
<15	476	4 49+3 68	
16-17	540	5 53+4 03	_
>18	85	7 35+4 40	22.650; 0.001
Schoo		7.55-1.10	
Vocational School of Health	103	4.41±3.89	
Sidika Hanim College	77	5.62±3.76	
Imam Hatip College	67	7.19±4.36	
SEV Anatolian College	142	3.97±3.57	
Zubeyde Hanim Girls' Vocational College	157	4.63±3.47	
Anatolian Vocational College	64	5.20±4.32	18.398; 0.001
Fahri Keskin Anatolian Teacher Training College	207	3.82±3.34	
Technical and Industrial Vocational College	284	6.92±4.05	
Class	5		
9.	368	4.69±3.73	
10.	317	5.27±3.40	
11.	227	5.32±3.91	
12.	189	6.06±4.42	5.073; 0.002
Resider	nce	1	
With family	650	5.43±4.09	-
Without family	451	4.92±3.82	2.098; 0.036
Presence of his/h	er own	room	
No	491	5.48±4.06	
Yes	610	5.01±3.92	1.944; 0.052
Family in	come		, , , , , , , , , , , , , , , , , , , ,
Bed	64	7.55±3.95	
Middle	775	5.15±4.01	
Good	262	4.86±3.78	12.334; 0.001
Type of Ch	aracter	r	
A type	579	5.54±4.17	
B type	522	4.87±3.76	2 705: 0 005
Total	1101	5.22±3.99	2.795, 0.005



Figure 1. Correlation of students' scores BHS and BDI

313) in our study. Of the students, 67 (6.1%) were overweight / obese. The Students' distribution of mean BHS scores according to some habits and medical properties is given in Table 2. The Students' distribution of mean BHS scores according to the some parental characteristics is given in Table 3. The mean score on BHS was 5.22 ± 3.99 , ranging from 0 to 19. The mean score on BDI was 13.59 ± 10.92 , ranging from 0 to 51. A significant positive correlation was found between the scores on BHS and BDI (rs = 0.343, p<0.001) (Figure 1).

Discussion

The hopelessness, which is known to cause many psychiatric disorders, particularly to depression and suicide, is a common health problem, es-

Table 2. Distribution of mean	n BHS scores accora	ing to some hab	oits and medical	properties of	f the students
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Some habits and medical properties	n	BHS Score X±Sd	Statistical analyses t/F; p			
Smoking						
No	973	4.83±3.79				
Yes	128	8.17±4.25	9.231; 0.001			
Chronicle	disease hist	ory				
No	1057	5.14±3.92				
Yes	44	7.16±5.08	3.301; 0.001			
Physic	al disability	•				
No	1011	5.27±3.97				
Yes	90	4.69±4.19	1.325; 0.186			
Mental	llness histor	у У				
No	1021	5.03±3.86				
Yes	80	7.74±4.72	5.946; 0.001			
History of prior adve	rse event af	fecting the life				
No	765	4.76±3.74				
Yes	336	6.28±4.33	5.900; 0.001			
Acn	e vulgaris					
No	464	5.05±3.95				
Yes	637	5.35±4.02	1.197; 0.231			
Overweight / obesity						
No	1034	5.11±3.92				
Yes	67	6.99±4.61	3.754; 0.001			
Depress	sion doubtfu	l				
No	788	3.96±3.05				
Yes	313	8.41±4.29	10.224.0.001			
Total	1101	5.22±3.99	19.324, 0.001			

pecially among adolescents. In this study, students' mean score on BHS was 5.22 ± 3.99 . Students' mean score on BHS was reported to be 6.18 ± 4.08 in the study of Ozmen et al. (6), 6.90 ± 3.40 in the study of Arslan et al. (16), and 10.07 ± 3.1 in the study of Tokuc et al. (27). The discrepancy of the results from these studies may be attributed to the socioeconomic and cultural differences between the populations studied.

Several studies (6, 17, 28) have found higher levels of hopelessness in men than women. In our study, the level of hopelessness was also significantly higher in boys than girls (p<0.05). This can be explained by the facts that the men living in the study area have more concern for the future than women and are primarily responsible for family's livelihood. However, extensive research is needed to make more accurate assessments. There are also some studies reporting no association between gender and the level of hopelessness (29, 30). As the adolescent grows older, the expectations of family and community from them will increase. The concerns about meeting these expectations may increase the level of hopelessness in the adolescent. In our study, the level of hopelessness among students significantly increased with the increasing age (p<0.05). Although similar results have been reported in other previous studies (31, 32), there are also some studies reporting no association between the age and level of hopelessness (17, 30). Among the colleges included to the study, students who were studying at Technical and Industrial Vocational College and at Religious Vocational College had a higher level of hopelessness than those studying at other colleges (p < 0.05). This result can be explained by that the students studying at vocational schools have less chance of accessing higher education due to the coefficient

			Statistical
		BHS Score	analyses
Some characteristics of parents	n	X±S0	t/F; p
Fam	ily type		
Nuclei	925	5.05±3.91	
Large	176	6.11±4.29	3.247; 0.001
Moth	er in life		
No	12	5.83±4.13	
Yes	1089	5.22±3.99	0.533; 0.594
Fath	er in life		
No	40	5.35±3.76	
Yes	1061	5.22±4.00	0.206; 0.837
Mother edu	ucational	level	
Under first school	119	6.61±4.60	
First-Middle school	855	5.06±3.86	0 110 0 001
College and upper	127	5.02±4.03	8.119; 0.001
Father edu	cational	level	1
Under first school	53	7.55±4.91	_
First-Middle school	644	5.15±3.87	0.705.0.001
College and upper	404	5.03±3.97	9.705; 0.001
Mother em	ploying s	status	1
No working	993	5.23±4.00	_
Working	108	5.13±3.96	0.255; 0.799
Father em	ploying s	tatus	
No working	203	5.52±4.14	
Working	898	5.15±3.96	1 105. 0 226
Total	1101	5.22±3.99	1.185; 0.236

Table 3. Distribution of mean BHS scores according to the some parental characteristics of the students

system implemented in the exam for transition to Higher Education. The level of hopelessness was low in the first class students and higher in the last year students (p<0.05). This can be attributed to having more concerns for the future and about the exam for transition to Higher Education. Tumkaya (31) also reported similar results in his study.

Due to several reasons such as mutual understanding on common issues and willingness to help between the peers as well as due to less family pressure about the future, the level of hopelessness is expected to be low among students living away from family home. Students who were staying with their parents in our study revealed higher levels of hopelessness (p<0.05). This can be explained by the facts that students who are living with their family feels more family and community pressure and they do not receive adequate support from their families. Morano and colleagues (33) also suggested higher levels of hopelessness in students living with the parents. The level of hopelessness reduces if students have their own room. However, we found no difference in the level of hopelessness between the students who had a private room and those who did not (p>0.05). This result might be resulted from the small number of students in the study who had their own room.

Adequately and timely unmet needs of the students due to a poor family income status are a major factor causing hopelessness (34). In our study, the level of hopelessness was significantly higher among students with poor family income (p <0.05). Similar results have been reported in some studies (6, 35).

The level of hopelessness was significantly higher in students with type A personality compared to those with type B personality (p<0.05). Some studies have also reported that individuals with type A personality are more hopeless (36, 37). Mental disorders are expected to be more common in adolescents who smoke. Accordingly, the level of hopelessness was found to be higher in students who smoked than those who did not (p<0.05). Similar results have also been reported in other studies (38, 39). Among the study participants, the level of hopelessness was higher in those with a history of any illness that requires continuous drug use than those without (p<0.05). This can be explained by the facts that necessity of continuous drug use negatively affects the quality of life and raises the concerns about the future. Deveci et al. (38) also found similar results in their study. Mental disorders such as depression, anxiety and hopelessness may be more common among individuals who are in need of support and protection due to physical disability. In our study, there was no difference in the level of hopelessness between students with and without physical disability (p>0.05). This might be resulted from the small number of students with physical disability in the study.

The individuals with a history of psychiatric disorder would be more susceptible to stress factors, they would create differences in coping with the existing problems and so, psychological processes would be affected (40). It was found that students with a history of prior mental illness had a higher level of hopelessness (p < 0.05). In their study, Celikel and Erkorkmaz (17) have reported similar results. Keller and colleagues (41) have reported that different types of life events manifest as different psychiatric disorders and all negative life events are especially associated with depressive symptoms. Similarly, we found higher level of hopelessness in students with a history of previously experienced events that adversely affect the life (p<0.05).

Psychologically vulnerable adolescents tend to be sensitive to their appearance and changes in their bodies (42). Acne can cause psychological distress and is associated with a large number of disorders. Halvorsen and colleagues (43) have reported that mental problems such as anxiety, depression and emotional isolation are relatively more common in adolescents with acne. In our study, there was no difference in the level of hopelessness between students who had acne on his/her face than those who did not (p>0.05).

The association of increased BMI with suicidal ideation, major depression, or other psychological problems is a well-known fact (44). In our study, the level of hopelessness was higher among overweight / obese students (p<0.05). Falkner et al (45) have reported similar results in their study.

It is also well-known that there is a strong positive correlation between depression and hopelessness (16, 46). In our study, the level of hopelessness was significantly higher in students who were suspected to have depression than those who were not (p<0.05). Some studies have also reported similar results (6, 18). Large number of individuals in a family causes problems in some fields such as housing, clothing, nutrition and education. It is clear that inadequate interest of parents in care of their children will adversely affect the children's mental health. Among the students included to the study, those with a patriarchal family type had a higher level of hopelessness than those with a nuclear family type (p<0.05). However, Kucuk and Arikan (47) found no relationship between the family type and level of hopelessness.

Inadequate social support, and lack of an individual who will represent the parents and can exhibit logical and consistent behaviors against the adolescents' positive and negative behaviors as well as with whom feelings and thoughts can be shared are known to be negative factors affecting the levels of hopelessness (30, 48). In this study, there was no difference in the level of hopelessness between the students whose parents were alive or dead (p>0.05). Tumkaya (31) have reported similar results in his study. Shek and Lee (49) reported a significant relationship between hopelessness of adolescent and educational level of parents. In our study, the level of hopelessness was significantly higher in students whose mother and father had not graduated from elementary school (for each, p < 0.05). Tumkaya (31), and Ozmen et al. (6) have reported similar results in their studies. This can be resulted from the fact that parents with a high education level show more interest in their children's education.

Kwok and Shek (50) have reported a relationship between parents' employment status and certain mental disorders in adolescents. We did not found such relationship between the level of hopelessness and the employment statuses of mother and father (p>0.05). In the study of Tumkaya (31), there was no relationship between the level of hopelessness and maternal employment status, whereas a higher level of hopelessness was reported among students whose father was not working.

Limitations

The major limitations of this study are that it was a cross-sectional study, included only the students who are studying in colleges of a single town, and that depression and hopelessness were not evaluated by precise diagnostic methods.

Conclusions and suggestions

Hopelessness is a major health problem among college students in Sivrihisar. This study found a significant positive relationship between hopelessness and depression. Efforts to produce a solution for changeable hopelessness-related factors and effective guidance services in schools to gain the ability of dealing with the existing problems can help to reduce the level of hopelessness. Furthermore, it was concluded that students with a high level of hopelessness and who were suspected to have depression should be referred to a psychiatrist for definitive diagnosis and treatment.

References

- 1. Beck AT, Lester D, Trexler M. The Hopelessness Scale. Journal of Consulting and Clinical Psychology. 1974;(42):861-74.
- 2. Velting DM. Personality and negative expectancies: trait structure of the Beck Hopelessness Scale. Personality and Individual Differences. 1999;26(5):913-21.
- 3. Dilbaz N, Seber G. Umutsuzluk kavramı: Depresyon ve intiharda önemi. [Turkish]. Crisis Journal. 1993; 1: 134-8.
- 4. Kashani JH, Soltys SM, Dandoy AC, Vaidya AF, Reid JC. Correlates of hopelessness in psychiatrically hospitalized children. Comprehensive Psychiatry. 1991;32(4):330-7.
- 5. Haatainen KM, Tanskanen A, Kylmä J, Honkalampi K, Koivumaa-Honkanen H, Hintikka J et al. Gender differences in the association of adult hopelessness with adverse childhood experiences. Soc Psychiatry Psychiatr Epidemiol. 2003;38(1):12-7.
- Özmen D, Dündar PE, Çetinkaya AÇ, Taşkın O, Özmen E. Hopelessness and factors affecting hopelessness in high school Students. Anatolian Journal of Psychiatry. 2008;9(1):8-15.
- Ceylan A, Özen Ş, Palancı Y, Saka G, Aydın YE, Kıvrak Y ve ark. Anxiety-depression levels and harmful habits at last year of high school students (the research of Mardin province). Anatolian Journal of Psychiatry. 2003;4:144-150.
- 8. Beck AT, Steer RA, Kovacs M, Garrison B. Hopelessness and eventual suicide: a 10-year prospective study of patients hospitalized with suicidal ideation. Am J Psychiatry. 1985;142 (5):559-63.

- 9. Cheung AH, Dewa CS. Mental health service use among adolescents and young adults with major depressive disorder and suicidality. La Revue Canadienne de Psychiatrie. 2007;52:228-32.
- 10. Brown GK, Beck AT, Steer RA, Grisham JR. Risk factors for suicide in psychiatric outpatients: a 20-year prospective study. J Consult Clin Psychol. 2000;68(3):371-7.
- 11. Moscicki EK. Identification of suicide risk factors using epidemiologic studies. Psychiatr Clin North Am. 1997;20:499-518.
- 12. Kashani JH, Reid JC, Rosenberg T. Levels of hopelessness in children and adolescents: a developmental perspective. J Consult Clin Psychol. 1989;57:5-7.
- 13. Everson SA. Hopelessness and risk of mortality and incidence of myocardial infarction and cancer. Psychosomatic Medicine. 1996;58:113-21.
- 14. American Psychiatric Association (2000) Diagnostic and Statistical Manual of Mental Disorders. DSM-IV-TR. Washington DC: American Psychiatric Association.
- 15. Mystakidou K, Tsilika E, Parpa E, Athanasouli P, Galanos A, Anna P et al. Illness-related hopelessness in advanced cancer: influence of anxiety, depression, and preparatory grief. Arch Psychiatr Nurs. 2009;23(2):138-47.
- 16. Arslan S, Celebioglu A, Tezel A. Depression and hopelessness in Turkish patients with cancer undergoing chemotherapy. Jpn J Nurs Sci. 2009;6(2):105-10.
- 17. Çelikel F, Erkorkmaz U. Üniversite Öğrencilerinde Depresif Belirtiler ve Umutsuzluk Düzeyleri ile İlişkili Etmenler. [Turkish]. Nöropsikiyatri Arşivi. 2008; 45: 122-9.
- Cunningham S, Gunn T, Alladin A, Cawthorpe D. Anxiety, depression and hopelessness in adolescents: A structural equation model. J Can Acad Child Adolesc Psychiatry. 2008;17:137-44.
- 19. Bentley KW. Predictors of suicide ideation, depression, and hopelessness in college and college students. Dissertation Abstracts International: Section B: The Sciences and Engineering. 1999;60(4-B):1841.
- 20. Beck AT, Weissman A, Lester D, Trexler L. The measurement of pessimism: the hopelessness scale. J Consult Clin Psychol. 1974;42(6):861-5.
- 21. Seber G, Dilbaz N, Kaptanoğlu C, Tekin D. Umutsuzluk ölçeği: geçerlilik ve güvenirlilik. [Turkish]. Kriz Dergisi. 1993; 1 (3): 139-42.
- 22. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. Arch Gen Psychiatry. 1961;4:561-71.

- 23. Hisli N. A study of the validity of the Beck Depression Inventory. Turkish J Psychol. 1998;6:118-22.
- 24. Green MS, Jucha E, Luz Y. Blood pressure in smokers and nonsmokers: epidemiologic findings. Am Heart J. 1986;111:932-940.
- 25. Friedman M. Type A Behavior: Its Diagnosis and Treatment. New York, Plenum Press (Kluwer Academic Press), pp. 31, 1996.
- 26. Li M, Yan H, Dibley MJ, Chang SY, Sibbritt D. Prevalence of overweight and obesity and its associated risk factors in students aged 11-17 in Xi'an in 2004. Zhongguo Yi Xue Ke Xue Yuan Xue Bao. 2006;28:234-9.
- 27. Tokuç B, Halil Evren SM, Ekuklu G. Hopelessness and Trait Anxiety Levels of Young Workers Attending to Occupational Training Center in Hayrabolu and Edirne [Turkish]. TAF Prev Med Bull. 2009;8(2):155-60.
- Haatainen KM, Tanskanen A, Kylmä J, Honkalampi K, Koivumaa-Honkanen H, Hintikka J et al. Stable hopelessness and its predictors in a general population: a 2-year follow-up study. Suicide Life Threat Behav. 2003;33(4):373-80.
- 29. Hamzaoglu O, Ozkan O, Ulusoy M, Gokdogan F. The prevalence of hopelessness among adults: disability and other related factors. Int J Psychiatry Med. 2010;40(1):77-91.
- Tanaka E, Sakamoto S, Ono Y, Fujihara S, Kitamura T. Hopelessness in a community population in Japan. J Clin Psychol. 1996;52(6):609-15.
- Tümkaya S (2005) Ailesi yanında ve yetiştirme yurdunda kalan ergenlerin umutsuzluk düzeylerinin karşılaştırılması. [Turkish]. Türk Eğitim Bilimler Dergisi. 2005;3(4):445-59.
- 32. Stoddard SA, Henly SJ, Sieving RE, Bolland J. Social connections, trajectories of hopelessness, and serious violence in impoverished urban youth. J Youth Adolesc. 2011;40 (3):278-95.
- *33.* Morano CD, Cisler RA, Lemerond J. Risk factors for adolescent suicidal behavior: loss, insufficient familial support, and hopelessness. Adolescence. 1993;28(112):851-65.
- 34. Patel V, Pereira J, Coutinho L, Fernandes R, Fernandes J, Mann A. Poverty, psychological disorder and disability in primary care attenders in Goa, India. Br J Psychiatry. 1998;172:533-6.
- Haatainen KM, Tanskanen A, Kylmä J, Honkalampi K, Koivumaa-Honkanen H, Hintikka J et al. (2004) Factors associated with hopelessness: a population study. Int J Soc Psychiatry. 2004;50(2):142-52.

- 36. Vatan S, Dağ İ. Problem solving style, hopelessness, helplessness and hapless-ness as the predictors of psychopathology assessed by MMPI-2. Anatolian Journal of Psychiatry. 2009;10(3):187-97.
- Buwaldo FM, Bouman TK, van Dujin MA. Psychoeducation for hypochondriasis: a comparison of a cognitive-behavioural approach and a problem-solving approach. Behav Res Ther. 2007;45 (5):887-99.
- 38. Deveci SE, Ulutaşdemir N, Açık Y. Bir mesleki eğitim merkezi öğrencilerinde umutsuzluk düzeyi ve etkileyen faktörler. [Turkish]. Dicle Tıp Dergisi. 2011;38(3):312-7.
- 39. Page RM, Zarco EP, Ihasz F, Suwanteerangkul J, Uvacsek M, Mei-Lee C et al. (2008) Cigarette smoking and indicators of psychosocial distress in Southeast Asian and Central-Eastern European adolescents. J Drug Educ. 2008;38(4):307-28.
- 40. Felsten G. Stress reactivity and vulnerability to depressed mood in college students. Pers Individ Dif. 2004;36:789-800.
- 41. Keller MC, Neale MC, Kendler KS. Association of differrent adverse life events with distinct patterns of depressive symptoms. Am J Psychiatry. 2007;164:1521-9.
- 42. Misery L. Consequences of psychological distress in adolescents with acne. J Invest Dermatol. 2011;131(2):290-2.
- 43. Halvorsen JA, Stern RS, Dalgard F, Thoresen M, Bjertness E, Lien L. Suicidal ideation, mental health problems, and social impairment are increased in adolescents with acne: a population-based study. J Invest Dermatol. 2011;131(2):363-70.
- 44. Goldney RD, Dunn KI, Air TM, Dal Grande E, Taylor AW. Relationships between body mass index, mental health, and suicidal ideation: population perspective using two methods. Aust N Z J Psychiatry. 2009;43(7):652-8.
- 45. Falkner NH, Neumark-Sztainer D, Story M, Jeffery RW, Beuhring T, Resnick MD. Social, educational and psychological correlates of weight status in adolescents. Obes Res. 2001;9(1):32-42.
- Kuo WH, Gallo JJ, Eaton WW. Hopelessness, depression, substance disorder, and suicidality-a 13-year community-based study. Soc Psychiatry Psychiatr Epidemiol. 2004;39 (6):497-501.
- 47. Küçük Y, Arıkan D. İşitme Engelli Çocukların Umutsuzluk Düzeylerinin Belirlenmesi. Uluslararası İnsan Bilimleri Dergisi. [Turkish]. 2005;2(2):1-13.
- 48. Gürvardar D. Yetiştirme Yurdunda Yetişen Çocuklar İle Anne Baba Yanında Yetişen Çocukların Umutsuzluk Düzeylerinin Karşılaştırılması. Yüksek Lisans Tezi. [Turkish]. Dokuz Eylül Üniversitesi Sosyal Bilimler Enstitüsü, İzmir, 2001.

- 49. Shek DT, Lee TY. Hopelessness in Chinese adolescents in Hong Kong: demographic and family correlates. Int J Adolesc Med Health. 2005;17(3):279-90.
- 50. Kwok SY, Shek DT. Socio-demographic correlates of suicidal ideation among Chinese adolescents in Hong Kong. Int J Adolesc Med Health 2008;20(4):463-72.

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Internalized stigma of elderly people's living in a nursing home from Turkey

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Abstract

Purpose: This study aims to analyze the internalized stigma experience of the elderly by investigating the subjective phenomenon defined by the elderly individuals living in a nursing home.

Method: This was a qualitative research. Phenomenological method was used to collect and evaluate the data. The research was conducted in a nursing home located in the Bornova district of İzmir province. The sample of the research consisted of 20 elderly individuals who were selected by homogenous and criterion sampling techniques. The elderly people who make up the sample were in-depth interviewed separately and face to face. The data were evaluated with Colaizzi's qualitative research analysis method.

Results: Five categories and seven themes were determined after the analysis of the interviews. Five categories were: (1) Perception of the relationship with society, (2) Views of the society regarding elderly individuals, (3) Family and environment attitude towards staying in a nursing home, (4) Opinions concerning other elderly individuals staying in a nursing home, (5) Perception of the present state. "Subjectivity" of the elderly staying in a nursing home should be protected and social support systems should be increased.

Conclusions: In this study, elderly people have shown various emotions to internalized stigma in nursing homes.

Keywords: Nursing home, old age, elderly, stigma, internalized stigma, qualitative study.

Introduction

Advances in medicine and technology cause life expectancy to be longer and the quality of life

to increase; consequently, resulting in an increase in the population of the elderly (1,2). The promotion of health and economic status in all over the world resulted in increasing the older population. It's expected, at least 40% of the population over 75 years, need extensive health care services in the last parts of their lives (3). Old age is the period in which individuals experience losses in terms of physical appearance, role and status, and become dependent on their environment due to loss of abilities and increase in physical disorders (4,5). World Health Organization (WHO) has acknowledged that old age is 65 and above. According to the statistics of 1992, 6.3 % of the world population is aged 65 and above. Projections on population indicate that 26 % of the European population is expected to be old aged in 2034 (6). The impacts of this demographic change manifest themselves as different problems in each society. As a result of fast industrialization and urbanization, transformation of agricultural order to urban and industrial order forces big families to be divided into nuclear families (7,8). The elderly member is isolated from the family; in a sense, pushed into loneliness. As a solution to these problems, societies have developed various structures, especially nursing homes and there has been an increase in the number of individuals living in nursing homes (9,10,11). Because, the urban environment is constantly changing and housing is replaced with nursing homes (12).

The number of elderly patients living in nursing homes rose substantially in the late 1980s and in the 1990s, resulting in a rise in workload for general practitioners (13). The elderly individuals living in a nursing home gradually lose the opportunity to know and control the environment that determines their self-acceptance and self-respect,

and the elderly individuals may experience feelings such as isolation and self-devaluation, and start isolating themselves and retreat from society (5,11,14). According to the findings of a research, almost one third of the elderly people living in a nursing home expressed that they experienced alienation from the outer world and that they were alone; two out of five stated that they were deprived of their own power of decision and one of every two individual stated that they were treated as "merely an elderly", adding that they were constantly reminded of this (9,11,13). Various researches support this phenomenon putting forward the fact that living in a nursing home is one of the major risk factors for isolation, self-depreciation and depression, causing severe psychological changes (4,9,11,13,14). These studies explored various perspectives of the resident's experience of nursing home life, and aimed at giving voice to the resident's views and improving their experience of life in the home. These studies include Gibb and O'Brien (1990) on social interaction between residents, Pearson et al. on staff mix and quality of life, and Gibson et al. (1992) on outcome measures (15). Nay (1995) studied the resident's perception of relocation (16), and Gorman (1996) studied the articulate resident's experience of living with other residents who express socially inappropriate behaviour (17). These studies explored nursing home residents' experiences, and searched for strategies to enhance the resident's quality of life.

One of the concepts that cause psychological change in elderly individuals living in a nursing home is "internalized stigma". Stigmatization is the act of disfavoring a person in the manner that s/he is discriminated, inferiorated, generally discredited (18) and emphasizing that certain groups are different. Goffman (1963) views processes of social construction as central; he describes stigma as "damaging social label, or attribute, that taints or discredits the person, influences social interactions, and jeopardizes opportunities for social inclusion (19,20). Newly, social scientists have elaborated the roles of labeling, stereotyping, cognition, and differential power and status in stigma (21,22,23). Horwitz (1978) argues that stigmatization is primarily caused by the person him/herse-If or their neighboring environment (24,25). The major source for the perception of stigmatization is the person him/herself (26). The first researches on stigmatization have focused on the stigma concerning various groups in peoples' minds; however, the subjective feelings and opinions of the stigmatized people on stigmatization have not been taken into consideration (22,25,27,28,29). Internalized stigma is the devaluation, shame, secrecy, and withdrawal resulting from the adaptation of negative stereotypes to oneself (19). The individual who experiences internalized stigma cannot feel that s/he owns a place s/he deserves in society or even belongs to that society (28,29). Internalizing the stigmatization in society severely weakens individuals (21,30). Although it is controversial whether social stigma or internalized stigma is more effective on the person, the stigmatization on people's minds is considered a more attainable target throughout the fight against stigmatization (31). Anxiety, depression, social isolation, decrease in self-esteem and hope are often observed in individuals who especially experience internalized stigma . Furthermore, individuals subject to all these are also under the threat of loss of social support, feeling of isolation, fear of exclusion and being abandoned (32,33). Internalized stigma is one of the outcomes of stigma, and it is thus an issue health professionals should assess in order to devise appropriate intervention plans towards its prevention (19).

Generally, Turkish society has a large family structure and the elderly peoples are valuable members of the family. In recent years, with the alteration of social values, the role of the elderly has changed its location within the family. Elderly peoples, who didn't seen needed attention for care by family, prefer to live in nursing homes. Therefore, the rate of transferring the elderly to the nursing home is increasing in Turkey too. Turkish society is still prejudiced this older people who lives in nursing homes. Turkish peoples think that the elderly peoples who lives in nursing homes undesirable by the families. Therefore they stigmatize them. Understanding elder's internalized stigma experiences is important for living in nursing home and understanding why this relocation causes that elder views it as "emotional experiences" is important, because this perceptions and fears have negative effects on elder's adaptation in countering with this challenge.

Purpose of the study

This study aimed to describe internalized stigma of elders living in nursing homes. The purpose of the phenomenological approach was to avoid generalizations and quantification and to understand the phenomenon as a human being experiences it.

Methods

Design

The qualitative phenomenological approach and purposive sampling amongst elderly residents governmental nursing homes in Turkey was applied.

Participants

The research was conducted at the a nursing home located in the Bornova district of İzmir province. Sampling continued till data saturation and resulting sample size became 20 participants. The population of the research consisted of individuals aged above 65 determined by individual reality. Elderly people included in the study we are could speak and understand Turkish, had be en on nursing home for at least one year, we are willing to participate in the study, had no physical and mental disabilities, and could express them selves comfortably.

Colaizzi's phenomenological methodology

The current study utilized a qualitative method, Colaizzi's method (1978) to approach the subject. According to this method, the researcher must first write a presupposition based on his/her own experiences in the area of interest, because "it was seen that the phenomenologist must initiate his [or her] inquiry by an examination of his approach in order to uncover his [or her] presuppositions about the investigated topic". Then, the researcher asks a few people about the phenomenon and adds new ideas to the original presupposition. Research questions are formulated based on this presupposition; the researcher interviews participants who have abundant experiences regarding the topic. The content of the interview is written down, and these interviews are analyzed in depth. Lastly, the researcher formulates a statement of identification of the fundamental structure of the investigated phenomenon (34).

Ethical issues

Researchers obtained permission from the Ethics Committee at one of University School of Nursing. *Social Assistance and Solidarity General Directorate*, Nursing Home and the elderly individuals who agreed to participate in the research. Verbal consent was obtained from each participant. Assurance of participant anonymity was given.

Data collection

The data of the research were collected with "Descriptive Characteristics Form" and "Semistructured Interview Form". Twenty elderly individuals were interviewed using semi-structured research questions. As for the semi-structured interview form, a total of ten questions were prepared and grouped under two titles, namely; "for living in society", "for the feelings experienced". The questions related to: nursing home experience, reasons for coming to nursing home, expectations of nursing home. To improve the reliability of the data and to facilitate a trusting relationship, one researcher conducted all the interviews. Data were collected using audio taped semi-structured interviews. Interviews were conducted in a private room on the nursing home and lasted between 30 and 60 minutes (45-minute interview on average). The data was collected with in-depth, open-ended interviews and participant observation and field notes. The interviews were conducted in a silent venue and not interrupted. During the interview, the interviewer and the interviewees were seated at the same level and the participants were actively listened to and guided with the questions. During the interview, noticeable situations were recorded as observation notes. Colaizzi's method was used for data analysis and the rigor was based on transferability and credibility. Descriptive characteristics form comprised of questions on sex, age, marital status, social security status and the length of residency at the nursing home.

Data analysis procedures

We reviewed all interview reports multiple times to analyze the data with the Colaizzi method of analysis (Table 1). We organized the formulated meanings in to categories and themes as described by Colaizzi (34). When differences existed between the investigators regarding the appropriate categories and themes, they were discussed until a consensus was reached.

Findings

When the socio-demographic data distribution of the elderly individuals were analyzed; it was determined that 50 % were male, 80 % were not married, 60 % had social security, all of them lived in the nursing home for three years and that this place was the first nursing home they lived in. Following the analysis of the data, the expressions made by the elderly individuals during the interviews were grouped under five categories. Five clusters of themes were identified. Consistent with the questionnaire format, five categories and seven themes of responses were determined describing the internalized stigma of the elderly persons (Table 2).

Categorie 1. Perception of the relationship with society

The majority of the elderly individuals perceive that their relationship with society is poor. They

express that they cannot develop a relationship with society or adapt to it and that they ended their relationship with society after they moved to the nursing home.

Theme 1. Poor communication

"My relationship with society is poor. I ended my relationship with society after I moved to the nursing home." My relationship with society is poor, because I am not conscious anymore, what use do I have for the society?." "I cannot build a relationship with society, because I feel excluded.

Categorie 2. Viewpoint of the society

All of the elderly individuals express that they are being in needy person, insulted and belittled by the society which has a negative impact on them and that they feel sorry. Moreover, they argue that society regards them as state-mandated and that they emphasize this fact as a negativity placed against them.

Theme 1. Needy people

"Society has negative views of us and we are being needy person, because the idea of nursing homes has not been embraced in our country yet. They tell me that I have a son and a daughter, but they have sent you to a nursing home. This situation really upsets me."

Table 1. The steps in Colaizzi's phenomenological data analysis

1. Read all the participants' description of the phenomenon under study.
2. Extract significant statements that pertain directly to the phenomena.
3. Formulate the meaning of these significant statements.
4. Categorize the formulated meanings into clusters of themes.
5. Integrate findings into an exhaustive description of the phenomenon being studied.
6. Validate the exhaustive description by returning to some of the participants to ask them how it
compares to their experiences.
7. Incorporate any changes offered by the participants into the final description of the essence of
the phenomenon.

Table 2. Categories and themes of internalized stigma of the elderly people that emerged from the interviews

Category	Themes
Perception of the relationship with society	Poor communication
Viewpoint of the acciety	Needy people
viewpoint of the society	Pitiful people
Attitude of family and environment towards living in a nursing home	Negative Opinion
Opinions concerning other alderly individuals living in a pursing home	Lonely people
Opinions concerning other elderry individuals riving in a nursing nome	Grief-stricken people
Perception of the present state	Unhappy people

Theme 2. Pitiful people

"Society has negative views of us and we are being needy person, because the idea of nursing homes has not been embraced in our country yet. They tell me that I have a son and a daughter, but they have sent you to a nursing home. This situation really upsets me." "They insult us. They say the state came to our aid and saved us. They say we would die otherwise. I'm trying not to pay attention, but I feel sorry anyway." "They feel pity for us. They think we are desolate and continuously console us. This situation makes me terribly sorry."

Categorie 3. Attitude of family and environment towards living in a nursing home

A significant majority of the elderly individuals expressed that the attitude of their families and environment was negative.

Theme 1. Negative Opinion

"They have a negative opinion about my being here." "My relations, especially my daughters are very angry with my staying here." "My friends are very angry with my staying here and ask me why I am living in a poorhouse."

Categorie 4. Opinions concerning other elderly individuals living in a nursing home

A great majority of the elderly expressed that they were sorry for and pitied the other elderly people, adding that they thought they were lonely.

Theme 1. Lonely people

"I think that some of them are lonely and desperate and I feel really sorry for them". "I really pity them."

Theme 2. Grief-stricken people

"I think they are sorrowful and grief-stricken. We are trying to develop an attachment with them. We are doomed."

Categorie 5. Perception of the present state

The elderly stated that they were not pleased with their present situation and that they suffered.

Theme 1. Unhappy people

"Thank God I'm healthy, but I'm very bitter." "There is jealousy among our friends, that's why I am neither peaceful nor happy." "I am unhappy, if only I weren't here.

Discussion

The aim of this phenomenological study is to grasp the internalized stigma experience of the elderly by investigating the subjective phenomenon defined by the elderly individuals living in a nursing home. Many examples are presented in order to demonstrate how the elderly experiences the perception of internalized stigma and how they cope with it. When the psychosocial changes that the elderly individual undergoes are considered, it is seen that the social activity and power, prestige, close relations, sexual activity, respectability, social life and support diminish, and a passive position replaces the former active roles. The individual who could once fulfill his/her own needs and help others gradually becomes a person who keeps consuming and is in need of help. At this stage, if the individual does not receive enough social support, he might remain vulnerable to the intense feeling of isolation caused by these phenomena (35).

Nursing homes are state or private institutions aimed at this idea, offering shelter, care and health control services for elderly individuals. However, many research determined that the elderly individuals living in a nursing home experience some psychosocial changes such as isolation from society, loneliness and depression (10,33). Furthermore, the perception of internalized stigma, which is intertwined with these psychological changes, is a previously neglected topic.

According to our research, due to the perception of internalized stigma, elderly individuals have a negative perception of their relationship with society and prefer not to establish a relationship. This situation causes elderly individuals to have a negative perception of society's viewpoint towards them. As a matter of fact, Hoffmann (1988) stated that, in the first phase, nursing home residents are considered people in need, and might become objects of the institution. According to the results of a research conducted by Narr (1998), approximately one third of the elderly living in a nursing home express that their relation to the outer world is broken off and that they feel lonely (36). Together with all the negative feelings the elderly

individual develops, s/he also adopts a negative attitude towards the nursing home s/he lives in and perceives the place merely as a shelter. In one of his expressions, Narr (1990) emphasized that a nursing home could be a "comfortable home under exceptional conditions" (36). As a result of these facts, the elderly individuals who have negative feelings towards themselves and the nursing home they live in also experience negative feelings for their environment and the other elderly people they live together in the nursing home and perceive themselves as unhappy and desperate because of their present situation. In fact, the pitiful words present in the expressions of the elderly people concerning the other elderly individuals are caused by the empathy they develop for themselves. The elderly individuals who perceive themselves as such also build a similar attitude towards the other elderly people. As a result of the research, it was determined that the elderly individuals had a poor relationship with society and that they often experienced the feelings of being excluded, inferiorated and belittled by society. The elderly individuals stated that they gave the decision of living in a nursing home for the reason that they did not want to become a burden for their families and that they felt pity and sorry for the other elderly people living in the nursing home. Furthermore, it was determined that they were pleased with the fact that they were not homeless and that they thought their friends living in the nursing home should appreciate the value of their present environment.

Conclusion

In this study, elderly people have shown various emotions to internalized stigma in nursing homes. Every elder, with different context and different history in life, described its uniqueness experiences. In addition, pay attention to social, financial, health supports and mental, spiritual and physical needs of elders should be met through whole care, because only attention to physical needs of them, denies hopeful, meaningful and purposive life in nursing homes. It is essential that nursing homes are provided with qualified labor force such as doctors, nurses, sociologists, psychologists, social service experts and managers in order to protect the "subjectivity" of the elderly.

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Contributions

Study Design: EAK, GHY, HO, LK Data Collection and Analysis: EAK, GHY Manuscript Writing: EAK, GHY, HO, LK

References

- 1. Hugman, R. Ageing and the care for elderly people in Europe. London, the Macmillan Press Ltd., Britain; 1994.
- 2. Meriç, M., Oflaz, F. Yaşlı bireylerin düşme yaşantısıyla ilgili algıları ve günlük yaşamlarına etkisi üzerine niteliksel bir çalışma. Turkish Journal of Geriatrics. 2007; 10(1), 19-23.
- 3. Salarvand S, Abedi HA, Hoseini H, Salehi S, Karimollahi M. The emotional experiences of elderly people living in nursing homes. Annals of General Psychiatry. 2008; 7(Suppl 1):151.
- Şahin, M., Yalçın, M. (2003). Huzurevinde veya kendi evlerinde yaşayan yaşlılarda depresyon sıklıklarının karşılaştırması. Turkish Journal of Geriatrics. 2003; 6(1), 10-13.
- Hacıhasanoğlu, R., Yıldırım, A. (2009). Erzincan huzurevinde yaşayan yaşlılarda depresyon ve etkileyen faktörler. Turkish Journal of Geriatrics. 2009; 12(1): 25 - 30.
- 6. WHO/OMS (1998). Fifty Facts From The World Health Report. http://www.who.org/whr/1999factse.htm.
- 7. Harrington, C. Does investor ownership of nursing homes compromise the gualite of care. American Journal of Public Health. 2001; 9 (9), 1452-1455.
- 8. Kıssal, A., Beşer, A. Yaşlı İstismar ve İhmalinin Değerlendirilmesi. TAF Prev Med Bull. 2009; 8(4), 357-364.
- 9. Onur, B. Gelişim Psikolojisi, Yetişkinlik, Yaşlılık ve Ölüm. In: Onur B (Ed): İmge Kitabevi, Ankara, 1995; 78-105.
- 10. Teresi, J., Abrams, R., Holmes, D., Ramirez, M., Eimicke, J. Prevalence of depression and depression recognition in nursing homes. Soc Psychiatr Psychiatric Epidemiol, 2001; 36 (12), 613-620.
- 11. Tufan, İ. Huzurevinde Yaşamak. In: Tufan İ (Ed): Modernleşen Türkiye'de yaşlılık ve yaşlanmak. Yaşlanmanın sosyolojisi Anahtar Kitaplar Yayınevi, İstanbul, 2003, pp 130-147.

- 12. Ekström M. Elderly people's experiences of housing renewal and forced relocation: Social theories and contextual analysis in explanations of emotional experiences. Housing Studies. 1994; 9(3).
- 13. Fahey, T., Montgomery, A.A., Barnes, J., Protheroe, J. Quality of care for elderly residents in nursing homes and elderly people living at home: controlled observational study. BMJ 2003; 326 : 580.
- 14. Abrams, R.C., Teresi, J.A., Butin, D.N. Depression in nursing home residents. Clin Geriatr Med. 1992; 8, 309-322.
- 15. Gibb H & O'Brien B. Jokes and reassurance are not enough: Ways in which nurses relate through conversation with elderly clients. Journal of Advanced Nursing 1990; 15(1): 1389 1401.
- Nay R. Nursing home residents' perception of relocation. Journal of Clinical Nursing 1995; 4:319 325.
- 17. Gorman L. I'm on the edge all the time: Resident's experiences of living in an integrated nursing home. Australian Journal of Advanced Nursing 1996; 13(3): 7 11.
- 18. Corrigan, P. The impact of stigma on severe mental illness. Cogn Behav Pract. 1998; 5, 201-22.
- 19. Yanos PT, Roe D, Lysaker PH. Narrative enhancement and cognitive therapy: a new group based treatment for internalized stigma among persons with severe mental illness. Journal of International Group Psychotherapy 2011; 61:576-95.
- Person, B., Bartholomewb, L.K., Gyapong, M., David, G., Addiss, D., Borne, B.V. Health-related stigma among women with lymphatic filariasis from the Dominican Republic and Ghana, Social Science & Medicine. 2009; 68, 30–38.
- 21. Link, B.G., Phelan, J.C. Conceptualizing stigma. Annual Review of Sociology. 2001; 27, 363-85.
- 22. Sartorius, N. Lessons from a 10-year global programme against stigma and discrimination because of an illness. Psychology, Health & Medicine. 2006; 11(3), 383–388.
- 23. Scambler, G. Sociology, social structure and healthrelated stigma. Psychology, Health & Medicine. 2006; 11(3), 288–295.
- 24. Haghighat, R. A unitary theory of stigmatization. Br J Psychiatry. 2001; 178, 207-215.
- 25. Schulze, B., Angermeyer, M.C. Subjective experiences of stigma. focus group study of schizophrenic patients, their relatives and mental health professionals. Soc Sci Med. 2003; 56, 299-312.
- Arslan, H., Şener, D.K. Spiritüalite ve konfor kavramlarının Meleis'in kavram geliştirme sürecine göre irdelenmesi. 52. Maltepe Üniversitesi Hemşirelik Bilim ve Sanatı Dergisi. 2009; 2(1), 1-8.

- 27. Taşkın, O. İçselleştirilmiş damga ve damgalanma algısı. In: Taşkın O (Ed): Damgalanma ve damgalanma. Turkuaz Bilişim & Bilgisayar & Yayıncılık, Ankara, 2007; 31-41.
- Ersoy, M.A., Varan, A. Ruhsal Hastalıklarda içselleştirilmiş damgalanma ölçeği Türkçe formu'nun güvenilirlik ve geçerlik çalışması. Türk Psikiyatri Dergisi. 2007; 18(2), 163-171.
- 29. Oran, N.T., Şenuzun, F. Toplumda kırılması gereken bir zincir: HIV/AIDS damgalanması ve baş etme stratejileri. Uluslararası İnsan Bilimleri Dergisi. 2008; 5(1), 1–16.
- Mak, W.W.S., Mo P.K.H., Cheung, R.Y.M., Woo, J., Cheung, F.M., Lee, D. Comparative stigma of HI-VAIDS, SARS and tuberculosis in Hong Kong. Social Science and Medicine. 2006; 63(7), 1912–1922.
- 31. Carlos, A.M., Lima, I.L, Lars, J., Wolfgang, R. Stigma and discrimination against elderly people with mental disorders in Europe. Int J Geriatr Psychiatry. 2003; 18, 679–682.
- 32. Kocabaşoğulu, N., Aliustaoğlu, S. Stigmatization. Yeni Symposium. 2003; 41(4), 190-192.
- *33.* Benek-Higgins, M., McReynolds, C.J., Hogan, E., Savickas, S. Depression and the elder person: the enigma of misconceptions, stigma and treatment. Journal of Mental Health Counseling. 2008; 30(4), 283–296.
- 34. Colaizzi PF. Psychological research as the phenomenologist views it. In: Valle R, King M,eds. Existential Phenomenological Alternatives for Psychology. Ist ed. New York: Oxford University Press; 1978. p. 48-71.
- 35. Sütoluk Z, Demirhindi H, Savaş N, Akbaba M. Adana huzurevlerinde kalan yaşlılarda depresyon sıklığı ve nedenleri. Turkish Journal of Geriatrics. 2004; 7 (3), 148-151.
- 36. Donaldson, J.M., Watson, R. Loneliness in Elderly People: An Important Area for Nursing Research. Journal of Advanced Nursing. 1996; 24, 952-959.

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Investigation of clinical values of secondary transurethral resection in carcinomas with noninvasive bladder transitional-celled

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Abstract

Purpose: The application of the second transurethral resection (TUR) in patients with noninvasive bladder tumor is a current topic because the residual tumor may remain after TUR. In this study, it was aimed to evaluate the clinical efficacy of the secondary TUR in the bladder cancers with non-invasive superficial transitional-celled, which was carried out 4 to 6 weeks after the first TUR.

Materials and methods: The data of 55 patients who were diagnosed with bladder tumor between August 2003 and April 2010 and who accepted the second TUR 4 to 6 weeks after the first TUR and who were applied the second TUR were retrospectively reviewed.

Results: Residual tumor was determined in 21 out of 55 cases(38%). In the histopathological evaluation which was carried out after the second TUR in our patients,3.6% advanced stage and 10.8% lower stage were detected. Residual tumor was determined in 21 out of 55 cases(38%). In the histopathological evaluation which was carried out after the second TUR in our patients, 3.6% advanced stage and 10.8% lower stage were detected. In the second TUR in patients whom the residual tumor was detected, it was found out that the diameters of the primary tumor before the first TUR were big and the primary tumor was with multiple focus.

Conclusions: For the determination of the appropriate treatment of the algorithm in patients with non-invasive bladder tumor, it was shown in this study that the second TUR provided better evaluation of clinical stage and the detection of residual tumor.

Keywords: Grade, residual tumor, stage, transurethral resection, treatment.

Introduction

Bladder cancer develops as a result of the malignant transformation of the bladder mucosa. The cancer cells of the mucosa of the transitionalcelled of the urinary tract can be implanted to the mucosa or may migrate to the other regions. Tumors which had been treated with local resection have resulted from many parts of the bladder and recurrence has confirmed this trend. That makes our separation difficult whether the residual tumor belongs to the first tumor which has been treated inadequately, or it has been as a result of the tumor migration or implantation or it has been formed as a result of the multifocal carcinogenesis (1).

Transurethral resection (TUR) provides the sufficient material for the pathologic evaluation of the tumor grade and stage. The grade and stage of the tumor are important because it directs the treatment and affects the prognosis in patients whose bladder tumors detected (2). Secondary TURs can better provide the control of the superficial tumors. At the same time, these applications facilitate staging and grading of the tumor. The mistakes which have made in staging of the tumor may cause the errors in the selection of appropriate treatment for the patient. In addition, the remaining of the residual tumor tissue as a result of incomplete resection is a significant risk factor in terms of the progression of the disease. Therefore, some researchers recommend that the recurrent TURs have been done in cases with superficial bladder cancer (3,4,5). The purpose of the second TUR is not only to detect residual tumors, but also to reduce the burden of the tumor before adjuvant therapy and to decide the appropriate option of the treatment for the patient according to the changing tumor staging. In this study, it has been investigated how the secondary TUR, which was carried

out 4 to 6 weeks after initial TUR, changed the clinical staging and how it affected the treatment in the superficial (noninvasive) transitional-celled bladder cancers.

Materials and methods

In this study, hospital records of 55 patients who were diagnosed with primary non-invasive bladder cancer between August 2003 and April 2010 at the Department of Urology of Faculty of Medicine at Yuzuncu Yıl University and who were applied the second time of TUR between 4 and 6 weeks were retrospectively reviewed. The ages and genders of the patients and the sizes and results of histopathological examination, focal numbers of the tumors, which were detected at the end of the first and second TUR were noted. In all of the patients, TUR was carried out under general anesthesia or regional anesthesia by using the monopolar resectoscope (Karl Storz ® - Germany) with 30-degree-optics and 24F continuous-flowed. TNM classification of WHO 2002 was used in the histopathological staging of the tissue samples, obtained as a result of TUR.

Statistical analysis

In the statistical evaluation, the focal numbers, sizes and results of the histopathological examination of the tumors which were detected at the end of the first and second TUR were compared by using the one-way analysis of the variance with one another (one-way ANOVA). Descriptive statistics were presented as counts and percentages. p-values of less than 0.05 were considered significant. These statistical analyses were performed using the SPSS (ver. 13) statistical program.

The criteria of the exclusion from the study

The cases, who had small one-focused lowgrade papillary tumor, and whose tumor pathology was invasive weren't taken to the study.

Results

55 cases were taken to the study. The ages of the patients changed between the ages of 22-95 (mean $60,38 \pm 16,9$). Of the cases, 8 were female (14.5%) and 47 were male (85.5%). The second TUR was applied to all of the cases an average of 4 to 6 weeks after the first TUR. Of the cases, 33 were single-focused (60%), 22 (40%) were multiple-focused in the first TUR. diameter of the tumor was larger than 3 cm in all of the cases. After the first TUR, the histopathological data of the cases with bladder cancer have been summarized at table 1 and the stage and grade changes of the histopathologic of the tumor in patients between the second TUR have been summarized at table 2. In the second TUR, while the residual tumor was detected in 21 of the cases (38.1%), the residual tumor wasn't detected in 34 of the cases (61.9%).) While the ratio of the residual tumor was 11/31 (35.4%) after the secondary TUR in Ta tumors, this ratio was detected as 10/24 (41.6%) in T1 tumors.

While the lesions of 13 cases, detected the residual tumor in the second TUR were multifocal, the single-focused lesion was seen in 8 patients in the first TUR. The ratio of the residual tumor in single-focused tumors was found as 24% but the ratio of the residual tumor in multifocal tumors was found as 59%. In all of the residual tumors detected in the second TUR, the diameter of the tumor was under 1 cm and these tumors were mostly two-focused. The residual tumors were, at the same time, localized in the field of the old resection.

The diameters of the primary tumor and multifocality data of the cases detected and not detected the residual tumor in the second TUR have respectively been shown in table 3. The diameters of the tumor and focal numbers in patients detected the residual tumor in the first TUR were significantly higher than the diameters of the tumor and focal numbers of the cases not detected the residual

Table 1. Stage and grade distribution of the cases after the first TUR

Grade of Primary TUR								
The Stage of Primary Tumor		Grade I	Grade II	Total				
	Та	28 (%50.9)	3(%5.4)	31(%56.3)				
	T1	20(%36.4)	4(%7.3)	24(%43.7)				
	Total	48(87.3)	7(%12.7)	55(%100)				

tumor in the first TUR (respectively p <0.001, P <0.05) (table 3).

Discussion

More than 90% of the bladder cancers are transitional-celled cancer; however, the rest are squamous-celled cancer or adenocarcinoma. Approximately 80% of the transitional-celled carcinoma are superficial tumors. 70% and 80% of them remain superficial as well as reoccure, but 20-30% of them deepens [1] . The first option in the treatment of the superficial bladder cancer is to be taken with TUR all of the visible lesions. TUR need both diagnosis and treatment in the bladder cancers (6,7). But it may not be possible to remove the tumor completely in every patient due to a lot of negative factors such as the bladder wall is thin, it includes the trabeculation, the size and location of the tumor is not appropriate and the presence of invisible intraepithelial or submucosal tumors (8). The ratio of the residual tumor after TUR has changed between 4% and 78% (3,9,10,11). In this study in the second TUR, the ratio of the residual tumor was found to be 38.1%. As the stage of the tumor increases, the ratio of the residual tumor detected in TUR has increased (3,4). Zurkirchen et al. have found out that the rate of the residual tumor in the second TUR was respectively 27% and 37% in the study that they carried out in 214 patients with Ta and T1 tumor (12). In their study published in 2003, Grimm et al. have found out 27% of the residual tumor in Ta tumors in 83 patients that the TUR was done for the second time and they have found out the residual tumor at the rate of 53% in T1 tumors (10). Klan et al. have reported the rate of residual tumor to be 43% in patients with T1 tumor after the second TUR (4).

Table 2. The distribution of histopathologic stage and grade changes

	The number of the patient(%)	The change of The stage and grade (The number of the patient)
The cases determined the histopathologic difference after the second TUR	10(%18)	
A- The changes of the stage determined after the second TUR	8(%14.5)	
Low staging in the second TUR	5(%9)	$T1G1 \rightarrow TaG1(5)$
High staging in the second TUR	3(%5.5)	$T1G2 \rightarrow T2G2 (2)$ T1G1 \rightarrow T2G1 (1)
B- The changes of the grade determined after the second TUR	2 (%3.5)	
Low grading in the second TUR	2 (%3.5)	TaG2→TaG1 (2)
High grading in the scond TUR	0	
The cases not observed the tumor in the second TUR	34 (% 62)	
The cases not determined the histopathologic difference after the second TUR	11 (%20)	

Table 3. The mean primary tumor diameters and multifocalities of the cases detected and not detected the residual tumor in the second TUR

Residual tumor							
	Determined (+)	Not determined (-)	D				
	n=21	n=34	1				
The diameter of the primary tumor (mm)	56.6	36.9	P<0.001				
The focal number of the primary tumor	2.82	1.82	P<0.05				

In this study, the ratio of the residual tumor in the secondary TUR was found to be 35% in Ta tumors but this ratio was found to be 41% in T1 tumors. In his/her study, Herr has reported that the secondary TUR was important to verify the complete resection of the primary tumor in patients with Ta and T1 tumor, to control the residual tumor, to change the new staging and treatment regime, to detect the silent muscle invasion (3).

In his/her study, Grimm has identified 5.3% of the advanced stage and 2.7% of the advanced grade in the second TUR and has found the muscle invasion at 4% of these patients [10]. In the study that Mersdorf et al. carried out, the advanced staging were detected at 14% of Ta tumors and 24% of T1 tumors after the second resection (9). Herr has found the advanced staging of these rates as 33% in Ta tumors and 28% in T1 tumors (3). These results have shown that the staging in patients whom the second TUR was done changed largely. Thus, the shape of the adjuvant therapy which will be applied to the patients is going to change.

In the study that Dalbagni et al. carried out, they have found more advanced stage than T1 at only 13% of the patients whom they carried out early cystectomy and the indications such as common tumor, the presence of multifocality, incomplete resection or common carcinoma insitu (13). In contrast, in the study that Dutta et al. carried out with 78 patients, they have shown that 37% of the patients who diagnosed with Ta or T1 pathology in the first resection had the invasive bladder cancer in their cystectomy specimens (14). In this study, the histopathologic difference was found at 18% of the patients between the first resection and the second resection. It was found that the tumor was located in a higher stage only at 5.5% of them in the second TUR. The treatment plan was changed by doing the radical cystectomy to these patients. The results of the study presented here have shown that the advanced stage of the tumor was detected at less rate in the second resection according to the literature. The reason for this may be because of doing a more radical resection in the first TUR. However, in patients detected the advanced stage in the second TUR, it is obvious that the regime of the treatment will change and this will have the contribution to the survival. In cases detected lower stage in the second TUR, authors have recommend to be more careful in the access to the regimes of the treatment which have a high comorbidity (8). In our view, this situation may lead to the third TUR of the indication for the patients.

The localizations of the cases detected the residual tumor have been evaluated in the study of Grimm et al. and it has been found that 19% of the residual tumor were in a different location while 46% of it were detected in the area of the primary tumor (10). It has also been reported in the other publications in the literature that the location of the residual tumor was the most frequently available in the previously resected area (4,5,9). The reason of the bladder cancer found in different locations in the second TUR may be the implantation or migration of the cancer cells. In this study, all of the tumors determined in the second TUR were found in the area of the old scar. Being carried out carefully and radically of the first TUR may also be reducing the risk of the migration or implantation of the tumor.

In patients who underwent only TUR and the second TUR, Grimm has determined five-year recurrence-free survival respectively as 40% and 63% and has recommended to be carried out the second TUR especially in patients with high-risk (10). While some authors recommend the implementation of the second TUR in medium-and high-risk patients with T1 tumor, others have recommend the second TUR for all of the patients with superficial bladder-tumor including the patients with noninvasive Ta tumor (4,15,16). A second resection may not provide all of the tumors to be resected as 100%, but reduces the likelihood of the remaining of the residual tumor and increases the effectiveness of the conservative intravesical therapy (8).

Algorithms have not been determined for the treatment and the diagnosis of the residual tumor since it was not clear that under which circumstances preferred the ways of the implantation, migration, or multifocality of the shape of the development of the residual tumor in the bladder cancer. The bladder tumor when first detected, it has been understood that the secondary TUR was important for the detection of the presence of the residual tumor, the identification of the phase changes and the regulation of the appropriate tre-

atment although the TUR carried out was very decisive in the prognosis of this cancer. Carrying out the second resection can reduce the mortality risk of the patients especially in those who have got a lot of tumor volume and the focal number.

References

- 1. Edward M. Messing, MD. Urothelial tumors of the urinary tract In Campbell's Urology. Eight Edition, 4th volume W.B. Saunders, USA 2002; pp 2723-2784
- 2. Heer, HW. Uncertainty and outcome of invasive bladder tumors. Urol Oncol. 1996; 2: 92.
- 3. Herr H. The value of a second transurethral resection in evaluating patients with bladder tumors. J Urol. 1999; 162: 74-76.
- 4. Klan R, Loy V, Huland H. Residual tumor discovered in routine second transurethral resection in patients with stage T1 transitional cell carcinoma of the bladder. J Urol. 1991; 146: 316-318.
- 5. Vögeli TA, Grimm MO, Ackermann R. Prospective study for quality control of TUR of bladder tumors by routine 2nd TUR. J Urol. 1998; 159(suppl): 143.
- 6. Milner WA. Results in the treatment of bladder tumours. J Urol. 1953; 69: 657.
- 7. Marberger H, Marberger M, Decristofero A. The current status of transurethral resection in the diagnosis and theraphy of carcinoma of the urinary bladder .Int Urol Nephrol. 1972; 4: 35-44. Acta oncologica Turcica. 2006; 39: 108-111.
- 8. Mersdorf A, Brauer A, Wolff JM. Second transurethral high risk superficial bladder cancer: a must? J Urol. 1998; 159 (Suppl): 143.
- 9. Grimm MO, Steinhoff C, Simon X, et al. Effect of routine repeat transurethral resection for superficial bladder cancer : a long term observational study. J Urol. 2003; 170: 433-437.
- Filbeck T, Roessler W, Knuechel R, et al. 5 aminolevulinic acid –induced fluorescence endoscupy applied at secondary transurethral resection after conventional of primary superficial bladder tumours . Urology. 1999; 53: 77-81.
- 11. Zurkirchen MA, Susler T, Gaspert A, et al. Second transurethral resection of superficial transitional cell carcinoma of the bladder: A must even for experienced urologist. Urol Int. 2004; 72: 99-102.

- 12. Dalbagni G, Herr HW, Reuter VE. Impact of a second transurthral resection on the staging of T1 bladder cancer Urology. 2002; 60 (5): 822-824.
- 13. Dutta SC, Shappell SB, Smith JA. Radical cystectomy in high risk non–muscle invasive transitional cell carcinoma: indications and outcomes. J Urol. 2000; 163 (Suppl): 149.
- 14. Jakse G, Algaba F, Malmström PU, et al. A second -look TUR in transitional cell carcinoma :why? Eur Urol. 2004; 45: 539-546.
- 15. Miladi M, Peyromaure M, Zerbib M, et al. The value of a second transurethral resction in evaluating patients with bladder tumours . Eur Urol. 2003; 43 : 241-245.

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Is the distribution of respiratory tract flora correlates with the microorganisms in the surrounding environment after tracheotomy?

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Abstract

Objective: To determine how the microorganisms in the surrounding environment affect the distribution of the bacterial flora in patients' respiratory tracts after tracheotomy.

Methods: Patients who were on a ventilatorassisted breathing after tracheotomy in the intensive care unit (ICU) were included in this study. Samples were taken from patients' respiratory tract and other locations as well as from the surface of medical equipment, hands of medical staff and room air at different time points after tracheotomy. The bacteria in those samples were cultured and identified.

Results: The rate of bacterial colonization and infection in the lower respiratory tract was 95% with an increasing number of bacterial species during the first 15 days after the tracheotomy. Klebsiella pneumoniae was the most common pathogenic bacterium in patients' sputum. More overall, the distribution of bacterial species in the patient's sputum was closely related to that in the medical equipment as well as in the patient's own body (p<0.05).

Conclusion: The peak infection incidence occurs about 10 days after the tracheotomy. The respiratory tract flora distribution correlates with that in the ventilator tube as well as the patient's oral cavity, pharynx and the surrounding area of tracheal incision. Antibiotic-resistant bacteria are the major causes of ventilator-associated pneumonia (VAP).

Keywords: Tracheotomy, respiratory tract, flora distribution, correlation.

Introduction

Tracheotomy is an emergency procedure to prevent asphyxia caused by obstruction of upper air passage or paralysis of respiratory muscles. It is also an important approach to prevent asphyxia in coma patients due to the deposition of respiratory mucus. However, tracheotomy is also one of the major causes of lower respiratory tract infection, which in turn, leads to prolonged hospitalization time, increased financial burden and increased mortality in the ICU. Therefore, it is important to investigate how the respiratory tract flora distribution is affected by the bacterial species in the surrounding environment. The result may provide critical information for the development of approaches that could significantly reduce the risk of tracheotomy-related lower respiratory tract infections. Thus could lead to a reduction in the overall incidence of hospital infection and an improvement of treatment result in patients under critical conditions.

After tracheotomy, the defensive mechanisms in the airways are partially damaged, and thus may expose the lower airways directly to pathogens in the outside world. The damage of protective barrier makes the bacteria easy to invade the lower respiratory tract and cause infections even death. Patrico Jimenez et al have reported that the incidence rate of pneumonia after tracheotomy is between 18-58%¹. In China, the lower respiratory tract infection rate after tracheotomy is 86-100%^{2,3}. The lower respiratory tract infection often occurs during the first 2 to 4 days after the tracheotomy, and it accounts for up to 48% of total infection-related mortality ³⁻⁵. A large number of bac-

terial species have been found to colonize in the respiratory tract after tracheotomy. Gram-negative bacteria account for 70-76% of them ²⁻⁵. The most common species that cause infections are Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae and Proteus mirabilis. Multiple infections account for 28.4% of infection cases ³⁻⁶. It is known that Pseudomonas aeruginosa, Acinetobacter baumannii, Vancomycin-Resistant Enterococci (VRE), Klebsiella pneumoniae, Gram-positive bacteria and fungi are the leading causes of nosocomially acquired opportunistic infections. In 1999, a study in Spain indicated that Acinetobacter baumannii had become the third leading cause, from the eighth leading cause of infection in ICU patients 7,8. It was detected in 8.2% of patients and widely spread in hospitals. In recent years, Staphylococcus infections are also on the rise in the hospitals, and Staphylococcus has become another major cause of hospital infection ⁹. It seems that the main bacterial species that cause hospital infections are in constant change. For example, Zheng et al reported that Vancomycin-Resistant Enterococci (VRE) has become the common cause of hospital infections today 10, while in the past, infections were mainly caused by the medical staff because about 40% of health care personnel had bacteria on their hands ¹.

There are many studies investigating tracheotomy related infections. However, in most studies, the bacteria are cultured and identified from sputum samples taken randomly after the tracheotomy. The bacterial species at different time points after tracheotomy in the patient's respiratory tract and in the surrounding environment are not compared. Therefore, it is difficult to determine whether the bacterial species on the surface of medical equipment, inserted tubes and medical staff's hands are identical to those cause lower respiratory tract infections. It is not clear whether the change of respiratory flora after the tracheotomy can be predicted and whether there is a correlation between the airway infection and the change of respiratory flora caused by tracheotomy.

In this study, we performed a dynamic monitoring of bacterial species in the patients' surrounding environment to determine whether they were related to the infections after tracheotomy. By examining the distribution of respiratory flora and the bacterial species in the surrounding areas, we hope our study will provide information for the prevention of lower respiratory tract infection and the improvement of patient care quality.

Materials and methods

Study population

The patients included in the study were those received a tracheotomy for ventilator-assisted breathing due to various causes in the ICU at a Grade A hospital in China between August 2007 and February 2008. Those patients were also given a score of 5 or 6 on the Glasgow Coma Scale (GCS). There were 14 males and 6 females. The mean age of those 20 patients was 54.68±9.30 years. The average hospitalization time was 21.3 days. Those data were obtained from patients' clinical records.

Methods

Bacterial samples were taken 1, 5, 10 and 15 days after the tracheotomy from 11 locations. Four samples taken from the patient were from sputum, oral cavity, pharynx and the site of tracheal incision. Five samples were taken from the surfaces of medical equipment, which included drainage tube, ventilator tube interface, humidification bottle of the ventilator and inside endotracheal tube. One sample was taken from the air of the room and the other was taken from the hands of medical staff. The bacteria in the samples were cultured and identified in the laboratory.

Statistical analysis

The results from 11 locations at each time point were categorized into 5 groups: sputum, hands of medical staff, air, medical equipment (drainage tube, ventilator tube interface, humidification bottle of the ventilator and endotracheal tube), and the patient's own body (oral cavity, pharynx and the site of tracheal incision). Data were analyzed with Epidata 3.1 software and statistical analyses were performed using Stata 9.0 software. Correlations were determined by the general statistical description, the chi-square test and Spearman rank correlation analysis.

Results

Lower respiratory tract infection after the tracheotomy

Figure 1 shows the rate of bacterial colonization and infection in the patient's lower respiratory tract after the tracheotomy. The incidence of infection was the highest 10 days after the tracheotomy due to a significant increase of new infection case on day 5 after the tracheotomy. This result indicated that most infections occurred within the first 10 days after the tracheotomy.

From Table 2, one could tell that there were 12 types of bacteria in the patients' sputum during the first 15 days after the tracheotomy. The distribution pattern of pathogenic bacteria on day 5, 10 and 15 after the tracheotomy showed that Klebsiella pneumoniae was the first appeared pathogen, while the antibiotic-resistant Pseudomonas aeruginosa and Staphylococcus aureus became the leading

bacterial species with the increasing of time. Table 3 shows that the bacterial species identified in the patient's sputum on day 5, 10 and 15 after the tracheotomy were closely related to those identified on the surface of medical equipment and the patient's own body based on the results of rank correlation analysis (p<0.05).

Discussion

Incidence of lower respiratory tract infection during the post-operative period in tracheotomy patients

Our result shows that the highest number of patient with a lower respiratory tract infection after the tracheotomy is on day 10, followed by day 15, day 5 and day 1. Most new cases of infection occur 5 days after the tracheotomy, and the number of new infection case starts to decrease after 10 days. This result is consistent with that has

Table 1. Low respiratory tract infection during the post-operative period in tracheotomy patients

Post-operative period (day)	Number of infected case	Number of no- infected case	Rate of infection (%)
1	1	19	5
5	12	8	60
10	19	1	95
10	17	3	85

Note: Yates correction $\chi^2 = 41.03$, P = 0.001.

Ten most common types of bacteria in the sputum during the post-operative period of tracheotomy

	Day 1		Day 5		Day 10		Day 15		
Rnk.	Bacterial species	Pos. (%)	Bacterial species	Pos. (%)	Bacterial species	Pos. (%)	Bacterial species	Pos. (%)	
1	K.pneumoniae	20	K.pneumoniae	55	K.pneumoniae	60	P. aeruginosa	60	
2	S.intermedius	20	P. aeruginosa 45		P. aeruginosa 60		K.pneumoniae	55	
3	A.baumannii	15	S. marcescens 20		S. marcescens 30		S.aureus	40	
4	S. marcescens	10	P.vulgaris	20	S.aureus	25	S. marcescens	40	
5	S.haemolyticus	10	S. intermedius	20	C.indologenes	20	P.vulgaris	20	
6	B. cepacia	10	C.indologenes	15	E.cloacae	15	C.indologenes	20	
7	M.morganii	10	S.aureus	15	S.intermedius	15	S intermedius	20	
8	P. aeruginosa	5	E.cloacae	15	A.baumannii	15	E.cloacae	15	
9	S.aureus	5	A. hydrophila	10	P.vulgaris	10	S.haemolyticus	10	
10	E.cloacae	5	S.haemolyticus	10	S.haemolyticus	10	A. hydrophila	10	
Р	0.747		0.005		< 0.001		0.001		

Table 2. Top ten bacteria identified in the sputum after the tracheotomy

Rnk - Rank Ps-Positivity

Note: Differences in positivity of identified bacteria were determined by the chi-square test. P values were calculated according to the Yates correction

Correlations between the distribution of bacterial species in patients' sputum and those in other locations after tracheotomy been reported by Xie et al ³, which shows that the number of new infection case starts to decrease 15 days after the tracheotomy. Therefore, the quality of patient care in the ICU during the first 10 days after the tracheotomy is critical to reduce the risk of hospital infection.

Gram-negative pathogens are the main cause of lower respiratory infection

The result from this study is consistent with those of others that show the lower respiratory infections are mainly caused by the Gram-negative bacilli⁴⁻⁶. However, within 15 days after the tracheotomy, the distribution of bacterial species changes along the time. The amount and positivity of bacteria that are more resistant to antibiotics and highly pathogenic gradually increase during the post-operative period. Among those pathogens, Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia marcescens and Staphylococcus aureus are the most abundant pathogens, and are the leading causes of lower respiratory infection during the first 15 days after the tracheotomy, while Klebsiella pneumoniae is the most common cause of infection during the first 10 days after the tracheotomy. Small amount of Acinetobacter baumannii has also been found in the samples collected on day 1 and 10 after the tracheotomy. A. baumannii was the leading cause of hospital infection in the past^{7,8}. However, the drug-resistant bacteria have become the main pathogens that cause infections nowadays. Therefore, it is important to monitor the bacterial species in the respiratory tract at different time points after the tracheotomy to determine when new bacterial colonies start to appear and how they are distributed among locations. This information is critical for the timely and appropriate treatment of post-operative infections to avoid a potential fatal outbreak of hospital infection.

Correlations between the bacterial species that cause lower respiratory tract infections after the tracheotomy and those in the surrounding environment

The bacterial species identified in the patient's lower respiratory tract 15 days after the tracheotomy are similar to those found on the surface of ventilator tube as well as in the patient's oral cavity, oral pharynx and the site of tracheal incision. However, unlike what have been reported previously⁵, the bacterial species that cause infections are different from those found on the hands of medical staff, suggesting that the quality of sterilization and infection control in the health care facilities has been improved significantly. As a result, the risk of infection caused by the medical equipment and staff has reduced in the ICU. A study conducted by Walther et al shows that the patients in an ICU with bedside hand washing facility use fewer amount of antibiotics than those without an access to such hand washing facility⁶. In recent years, the incidence of infection caused by medical staff in the ICU has decreased significantly due to the high sterilization standard.

Limitation

One limitation of this study is that the DNA sequences of those bacteria found in different locations were not analyzed. Therefore, the origin of those bacteria found in the patient's respiratory tract could not be determined.

Post- operative period (day)	Correlation coefficient	Hands of medical staff	Room air	Medical equipment	Patient's own body	Skin (pre-tracheotomy)
Day 1	Spearman's	-0.2946	-0.0156	0.0296	0.2098	-0.2157
Day I	P-value	0.4086	0.9658	0.9353	0.5608	0.5496
Dars 5	Spearman's	0.416	-0.161	0.7646	0.8232	
Day 5	P-value	0.2318	0.6567	0.01	0.0034	
Day 10	Spearman's	0.0887	-0.1132	0.7524	0.6113	
Day 10	P-value	0.8076	0.7555	0.0121	0.0404	
Day 15	Spearman's	0.2862	0.5981	0.7658	0.6518	
Day 15	P-value	0.4228	0.0678	0.0098	0.0411	

Table 3. Rank correlations between the distribution of top ten bacteria in the sputum and those in the other locations during post-operative period of tracheotomy

Conclusion

The bacterial species that cause lower respiratory tract infections after the tracheotomy are closely related to what are found on the surface of ventilator tube as well as in the patient's oral cavity, oral pharynx and the site of tracheal incision. More importantly, during the postoperative period, the number of bacteria that are more resistant to antibiotics and pathogenic increases over the time, and these bacteria become the main pathogens causing ventilator-associated pneumonia (VAP) after tracheotomy.

Based on the respiratory flora distribution and species causing postoperative infections, it is recommended that hospitals should establish an ICU infection control committee to develop standard operation protocols (SOPs) for staff training, periodic risk assessment, and dynamic respiratory tract flora monitoring, thereby, preventing infections caused by a variety of risk factors and increasing the patient safety and success rate of tracheotomy.

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Reference

- 1. Jimenez P. et al. Incidence and Etiology of pneumonia acquired during mechanical ventilation .Crit Care Med.1989; 17: 882.
- Jiang QZ, Wang WM., Qian YY., Lower respiratory infections after tracheotomy. Chinese Journal of Nosocomiology. 1999; 9 (1): 26.
- 3. Xie MS, Hao JC, Lui GZ et al. Control of lower respiratory tract infection in traumatic brain injury patients with tracheotomy. Chinese Journal of Nosocomiology. 2005; 15(5):513-515.
- Huang WZ, Ke SY, Chen C, Analysis and culture of bacteria in sputum samples from tracheotomy patients. Journal of Practical Medical Techniques. 2005; 12 (10): 2712-2713.
- 5. Ismesel NA. Colonization intensive care unit patients by Pseudomonas aenuginosa. J Hospi Infact. 1993; 25(4):279.

- 6. Hou Z. The distribution and drug sensitivity of pathogens in tracheotomy patients and in endotracheal tubes. Laboratory medicine. 2006; 21(1):28.
- Vaque J, Rossello J, Trilla A, et al. Nosocomial infections in results of five nationwide seria preva lence survey. Nosocomial Infection Prevalence Study in Spain. Infect Control Hosp Epidemiol. 1996; 17:293-297.
- 8. Rello J. Acinetobacter baumannii infections in the ICU: Customization is the key. Chest. 1999; 115:1226-1229.
- Li L, Liu ST, Wei HE, Culture and drug sensitivity analysis of bacteria in the sputum of severe coma patients after tracheotomy. Guangzhou pharmaceutical. 2004; 35 (2):9-11.
- 10. Zheng JS, Lai SY, Chen MX, et al. Lower respiratory tract infection after tracheotomy. Chinese Journal of Nosocomiology. 2002; 12 (8): 571-573.
- 11. Fraser VJ, Jones M, Dundel J, et al. Candidemia in a tertiary care hospital Epidemiology, risk factors and predictors of mortality. Clin Infect Dis. 1992; 15: 414.

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Level of knowledge about vitamin D among students at a university in western Turkey: a retrospective study

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Abstract

Background: Vitamin D is of great importance in maintaining health in every age group. Its deficiency leads to great health problems. The objective was to determine the level of knowledge about vitamin D and its relationships among health students

Methods: This survey is a descriptive study conducted among the students studying at a School of Health, western Turkey, between 28 February-28 May 2011. Vitamin D knowledge of those who obtained 23 and over scores as a result of the ROC analysis were considered to be knowledgeable. Obtained data were evaluated by percentages ratios, Chi-square (x2) and t tests, with a p value lower than 0.05 taken as significant.

Results: While the knowledge about vitamin D in those studying at nursing department was the highest (67.6%) according to the other departments (46.2%) (p<0.05). The level was higher in those studying in the last class than those studying at the first class (51.1% and 78.0%, respectively) (p<0.001). The proportion of those whose knowledge was sufficient was 62.9%. The knowledge level in students who received knowledge previously about vitamin D was sufficient was the most (82.9%), whereas it was the lowest in those without (50.6%) (p<0.01). The question which was the most accurately known by the students was the phrase "Vitamin D is important in bone formation and growth" with a proportion of 91.6%, and the most wrongly known question was the phrase "the amount that an adult individual should take daily (96.3%)".

Conclusion: The low level of knowledge indicates that the importance of the issue during students' training is not highlighted enough. Training

should be relevant to the issues at all stages of education.

Keywords: Vitamin D, knowledge, students, deficiency.

Introduction

Vitamin D is a lipid-soluble steroid hormone which is synthesized from precursor chemicals in the skin when exposed to ultraviolet light, and which shows the effect on tissues with feedback mechanism (1). Since sunlight plays an important role in the synthesis of vitamin D, the level of vitamin D in the body shows seasonal changes (2). Vitamin D is responsible for bone formation and growth (3), and direct and indirect cell proliferation (1). The risk groups for vitamin D deficiency include pregnant women, infants, adolescents and older people (4,5). Short-height, rickets, rachitism, osteomalacia, osteopenia, osteoporosis, osteoporotic fractures, cardiovascular diseases such as heart attack, Type 1 diabetes, autoimmune diseases such as multiple sclerosis, infectious diseases, schizophrenia and some types of cancer, although many of which are in the stage of hypothesis, some others are well established, are known to be correlated with vitamin D deficiency (6,7). Due to all these, people with vitamin D deficiency are at greater risk than normal population for morbidity and mortality (8).

Vitamin D deficiency, highly prevalent condition, is a present in approximately 30% to 80% of the general population in studies conducted out in various countries (9-11). Similarly, the rate of vitamin D deficiency ranges between 25% - 75% also in Turkey's overall population (12-14).

In diseases associated with vitamin D deficiency, the quality of life of individuals is adversely affected, and the deficiency predisposes individuals to diseases, preventing activities of daily living (15). Adequate knowledge of vitamin D is very important in the prevention of diseases, and in the achievement of early diagnosis, treatment and rehabilitation.

This study was conducted to determine the level of knowledge about vitamin D and its relationships among students at a university of western Turkey.

Methods

This survey is a descriptive research conducted on the students studying at the Osmangazi University School of Health, western Turkey, between 28 February 2011 and 28 May 2011. At the school, a total of 630 students (517 female students, 82.1%; 113 male students, 17.9%) were receiving education during the study. At the School of Health with an education and training period of 4 years, education is provided in 3 departments including Nursing (n=387), midwifery (n=193) and Health Care Management (n=50). Of the 630 students, 226 were excluded from the survey due to: unwillingness to participate in the research (n=32), and not being at classes at the time of the study (n=194). The remaining 404 (64.1%) students (355 females, 87.9%; 49 males, 12.1%) constituted the study group. After distributing the questionnaires to students at the school, they were informed of how questionnaires were to be filled in, and then they were requested to make their choice applicable to themselves. The students completed questionnaires and inventories in the presence of a member of the research team. The data collected was self-reported by the students. All subjects were told that participation in the investigation was strictly voluntary, and that the data collected would not be used for anything except for this research study, and then they were given the questionnaires and inventories to complete. The duration for completing the questionnaire and inventory was between 30 and 35 minutes per subject.

Permission for the study was obtained by making a petition prior to collecting data. This was achieved by contacting and receiving approval from the Director of the Institution of Eskisehir Osmangazi University. The questionnaire, prepared with reference to previous studies in the literature included two parts. In the first part of the questionnaire, students were asked to state their socio-demographic characteristics, and the second part of the questionnaire included 50 questions of knowledge related to vitamin D. The student allowances were grouped as 'enough', 'medium', or 'insufficient' in the students' own statements, and the students' parents were divided into 2 groups: those who were actively working in any job (workers, civil servants, farmers, freelancers) and those who were not working (unemployed, retired, housewife).

Fifty questions of knowledge related to vitamin D in the questionnaire were subjected to scoring. While evaluating knowledge questions about vitamin D, each correct answer was given 1 point. The scores that students received from knowledge questions ranged from 0 to 50. Students' scores were divided into two sets by the K-means clustering analysis firstly. Then, ROC analysis was performed by reference to the cluster properties. Vitamin D knowledge levels of those who obtained 23 and over scores as a result of the ROC Analysis were considered to be adequate.

The statistical package for social sciences (SPSS) version 10.0 (Chicago, IL, USA) was used to enter and analyze the data on a personal computer. Obtained data were evaluated by frequency and percentages ratios, Chi-square (x2) and t tests. The measure for statistical significance was established a priori as P < 0.05.

Results

The mean (\pm SD) age of the respondents (n=404) was 20.86±1.80, ranging from 17 to 34 years. More respondents (87.9%) were females, and most of them (61.9%) were studying at the department of nursing, and the least at the department of management of health institutions (6.4%). Many of the students were from the 2nd class and less from the 4th class. Most students had graduated from public high school (37.4%). Nearly 50% (46.3%) were remaining at the dormitory, and had lived in a nucleus family type (85.9%). Most students' family income status was middle level (66.1%), and the number of students who were lack of health insurance was only 37 (9.2%).

Only 20% of the respondents reported that they had worked in a job with income, and only 5% of them were working during the study. While the level of knowledge about vitamin D in those studying at nursing department was the highest (67.6%), it was the lowest in those studying at management of health institutions (46.2%) (p<0.05). The level of knowledge about vitamin D was higher in those studying in the last class than those studying at the first class (78.0% and 51.1%, respectively) (p<0.001).

The level of knowledge about vitamin D showed increase as age increased; the level of knowledge was only 46.5% at the age of 19,

Table 1. Distribution of students with/without sufficirent level of knowledge about vitamin D by some sociodemographic characteristics

Some characteristics	Level of k	Vitamin D	Test value	
	Incuttoriant	Tatal	x²; p	
	Insumcient	Sumcient	Iotai	
	n (%)	n (%)	n (%)	
Department	01 (20 4)	1(0((7)))	250 ((1.0)	7.270 0.025
Nursing	81 (32.4)	169 (67.6)	250 (61.9)	/.3/8; 0.025
Widwifery	55 (43.0)	/3 (5/.0)	128 (31.7)	
Management of health	14 (53.8)	12 (46.2)	26 (6.4)	
institutions	1. (00.0)	12 (1012)		
Class				
1-2	111 (48.9)	116 (51.1)	227 (56.2)	30.748; 0.000
3-4	39 (22.0)	138 (78.0)	177 (43.8)	
Graduated high school				
Health vocational high school	11 (29.7)	26 (70.3)	37 (9.2)	1.129; 0.569
Public high school	72 (38.9)	113 (61.1)	185 (45.8)	
Anatolia high school	67 (36.8)	115 (63.2)	182 (45.0)	
Age groups				
19 years old and under	46 (53.5)	40 (46.5)	86 (21.3)	18.854; 0.000
20	44 (41.9)	61 (58.1)	105 (26.0)	
21	27 (31.4)	59 (68.6)	86 (21.3)	
22 years old and over	33 (26.0)	94 (74.0)	127 (31.4)	
Gender				
Woman	130 (36.6)	225 (63.4)	355 (87.9)	0.170; 0.680
Man	20 (40.8)	29 (59.2)	49 (12.1)	
Resting place				
With his family	32 (38.1)	52 (61.9)	84 (20.8)	2.410; 0.492
With friends at home	45 (39.5)	69 (60.5)	114 (28.2)	
Alone at home	4 (21.1)	15 (78.9)	19 (4.7)	
At dormitory	69 (36.9)	118 (63.1)	187 (46.3)	
Type of family				
Nucleous	125 (36.0)	222 (64.0)	347 (85.9)	1.673; 0.433
Patriarchial	19 (46.3)	22 (53.7)	41 (10.1)	
Fragmented	6 (37.5)	10 (62.5)	16 (4.0)	
Income status of family				
Poor	14 (50.0)	14 (50.0)	28 (6.9)	3.764; 0.152
Middle	102 (38.2)	165 (61.8)	267 (66.1)	
Good	34 (31.2)	75 (68.8)	109 (27.0)	
Health insurance				
Absent	14 (37.8)	23 (62.2)	37 (9.2)	0.000; 1.000
Present	136 (37.1)	231 (62.9)	367 (90.8)	
Working status	· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	
Not working	143 (37.2)	241 (62.8)	384 (95.0)	0.000; 1.000
Working	7 (35.0)	13 (65.0)	20 (5.0)	Í
Total	150 (37.1)	254 (62.9)	404 (100.0)	

whereas it increased to 74.0% at the age of 22 (p < 0.001). The schools that students graduated, working or not working at a income-generating job, family income status, the places where students stayed, those having/not having any social insurance as well as gender differences did not show a feature in terms of the level of knowledge about vitamin D (p>0.05, per one). The proportion of students whose mothers had an education level of primary school and lower was 66.8%, with the figure of 38.9% reported for students' fathers. The proportion of students' mothers working in a job with income was less (11.6%), with the proportion of 67.8% reporting that their fathers' working proportion in any job was 67.8%. Table 1 shows more distribution of students with/without sufficient level of knowledge about vitamin D by some sociodemographic characteristics.

Table 2 indicates the distribution of those with and without sufficient level of vitamin D by some characteristics. The proportion of those whose level of knowledge was sufficient was 62.9%. The level of knowledge in students with previous history of getting knowledge about vitamin D was higher when compared to those without (66.3% and 53.3%, respectively) (p<0.05). Those who received knowledge previously about vitamin D had the most level of knowledge (82.9%), whereas it was the lowest in those without (50.6%) (p<0.01). It did not reveal any difference in terms of sufficiency status of knowledge received previously about vitamin D in those who had and those who did not have a family history of using vitamin D (p>0.05). The source of knowledge in those with sufficient knowledge level was from written-verbal media at the least rate (58.5%). However, there was no difference in terms of source of knowledge received about vitamin D between those whose level of knowledge was sufficient and those whose level of knowledge was insufficient (p>0.05). The number of the students who reported that they had taken D vitamin for any reason was 19 (4.7%). Most students indicated that they had knowledge about D vitamin, and many students (60.3%) reported receiving much of knowledge from health subjects at schools. The proportion of students who noticed that they had sufficient level of knowledge about vitamin D was only 11.8%, followed by a proportion of 58.9% having a partly sufficient level of knowledge. The question which was the most accurately known by the students was the phrase "Vitamin D is important in bone formation and growth" with a proportion of 91.6%, and the most wrongly known question was the phrase "the amount that an adult individual should take daily (96.3%)". More detailed data are presented in Table 3.

Some characteristics	Knowled	Test value x ² ; p		
	Insufficient n (%)	Sufficient n (%)	Total n (%)	´ •
Family history of using vitamin D				
No	143 (37.1)	242 (62.9)	385 (95.3)	0.000; 1.000
Yes	7 (36.8)	12 (63.2)	19 (4.7)	
Previous history of getting knowle	edge about vitamir	n D	· · · ·	
No	50 (46.7)	57 (53.3)	107 (26.5)	5.747; 0.017
Yes	100 (33.7)	197 (66.3)	297 (73.5)	
If yes, source of knowledge receiv	red about vitamin	D		
Health personnel	12 (30.8)	27 (69.2)	39 (13.1)	3.335; 0.343
Written-verbal media	17 (41.5)	24 (58.5)	41 (13.8)	
Health information subject	65 (36.3)	114 (63.7)	179 (60.3)	
Sources more than one	9 (23.7)	29 (76.3)	38 (12.8)	
Sufficiency status of knowledge re	eceived previously	about vitamin	D	
Insufficient	43 (49.4)	44 (50.6)	87 (29.3)	14.231; 0.001
Sufficient	6 (17.1)	29 (82.9)	35 (11.8)	
Partly sufficient	54 (30.9)	121 (69.1)	175 (58.9)	
Total	150 (37.1)	254 (62.9)	404 (100.0)	

Table 2. Distribution of those with and without sufficient level of vitamin D by some characteristics

Propositions about vitamin D	True	False	No idea
-	n (%)	n (%)	n (%)
* Vitamin Dia a watar asluhla vitamin	70(172)	307	27 (67)
* Vitamin D is a water-soluble vitamin	/0(1/.3)	(76.0)	27 (0.7)
Sources of vitamin D are cholecalciferol and ergocalciferol	145 (35.9)	32 (7.9)	227 (56.2)
Vitamin D3 is synthesized by procedures in skin, liver and kidney	141 (34.9)	14 (3.5)	249 (61.6)
Oil and bile increases the absorption of vitamin D	261 (64.6)	39(97)	104(257)
Excess vitamin D is stored in the tissues such as lung fat and so		104	
on	183 (45.3)	(25,7)	117 (29.0)
Vitamin D is important in hone formation and growth	370 (01.6)	(23.7) 13(3.2)	21 (5 2)
Vitamin D is important in oone formation and growin	281 (69.6)	$\frac{13}{37}(92)$	$\frac{21}{86}(21.3)$
	201 (09.0)	$\frac{37(9.2)}{168}$	00(21.5)
* Excess calcium intake reduces the absorption of vitamin D	104 (25.7)	(100)	132 (32.7)
	~ /	(41.0)	<u> </u>
Vitamin D is abundant in fish oil and butter	170 (42.1)		123 (30.4)
		(2/.5)	
* Broccoli is a food-poor in Vitamin D	51 (12.6)	95	258 (63.9)
	51 (12.0)	(23.5)	200 (00.7)
Egg volk and liver are rich in vitamin D	183 (45 3)	72	1/10 (36.0)
	165 (45.5)	(17.8)	149 (30.9)
Nutriente de not include emergente of vitamin D to most the need	1(2(40.1))	127	115 (20 5)
Nutrients do not include amounts of vitamin D to meet the need	162 (40.1)	(31.4)	115 (28.5)
People should wait in the sun with open arms and legs15-20	2 (0 (01 0)		24 (5.0)
minutes each day	369 (91.3)	11 (2.7)	24 (5.9)
Synthesis of vitamin D varies according to the seasons	316 (78.2)	33 (8 2)	55 (13.6)
Geographical location and time of day affects vitamin D synthesis	$\frac{310(70.2)}{303(75.0)}$	$\frac{33(6.2)}{28(6.9)}$	73(18.0)
Open colour clothes affects adversely the synthesis of vitamin D	$\frac{303(75.0)}{310(76.7)}$	$\frac{20}{39}(9.7)$	55(13.1)
open colour cloules anecus adversery the synthesis of vitamin D	510(70.7)	$\frac{37(7.7)}{03}$	33 (13.0)
Sunscreen have a negative effect on the synthesis of vitamin D	175 (43.3)	(22.0)	136 (33.7)
	~ /	(23.0)	<u> </u>
Air pollution affects adversely the synthesis of vitamin D	154 (38.1)	$\frac{J}{(14.1)}$	193 (47.8)
F	- ()	(14.1)	
Those with dark skin should stay in the sun for a long time	168 (41.6)		119 (29 5)
	100 (11.0)	(29.0)	11) (2).0)
* to prevent skin cancer, vitamin D should be preferred instead	120 (29 7)	162	122 (30.2)
of sun	120 (2).7)	(40.1)	122 (30.2)
Vitamin D can be added into basic feedstuffs such as milk	106(48.5)	51	157 (28.0)
Vitaliili D call of added lifto basic loodstulls such as lillik	190 (40.3)	(12.6)	137 (30.9)
Vitamin D deficiency is common in alcohol and drug abusers	172 (42.6)	22 (5.4)	210 (52.0)
The use of antiepileptics may be resulted in rickets / osteomalacia	172 (42.0)	20(50)	211 (52 2)
may result	1/3 (42.8)	20 (5.0)	211 (52.2)
The need for vitamin D in pregnant women is 3-4 times higher	244 (05.1)		52 (12.0)
than in adults	344 (85.1)	8 (2.0)	52 (12.9)
Vitamin D requirement of the fetus is provided from mother's			
stores	327 (80.9)	14 (3.5)	63 (15.6)
Pregnant women's benefiting from the sun is important for the			
for the sum of the source of the sum of the	349 (86.4)	10 (2.5)	45 (11.1)
There is a sufficient emount of vitamin D for the holes in	~ /	124	<u> </u>
There is a sufficient amount of vitamin D for the baby in	179 (44.3)	134	91 (22.5)
mother's milk	()	(33.2)	
Infant formulas include sufficient amounts of vitamin D	51 (12.6)	220	133 (32 9)
	• • • (•=••)	(54.5)	100 (02.3)
Vitamin D drug support should be given to all newborns	211 (52.2)	71	122 (30.2)
The indicated by the in	<u> </u>	(17.6)	122 (30.2)
Vitamin D is free medications to infants in Turkey	148 (36.6)	24 (5.9)	232 (57.4)
400 IU / day vitamin D supplements should be given to babies	168 (41.6)	12 (3.0)	224 (55.4)
* In adults, vitamin D requirement is at least 2000 IU/day	89 (22.0)	26 (6.4)	289 (71.5)
Need for vitamin D increases in women entering menopause	310 (76.7)	15 (3.7)	79 (19.6)
Vitamin D deficiency is common in the elderly	343 (84.9)	8 (2.0)	53 (13.1)
Elderly should take oral vitamin D dose of 2x10.000-100.000 IU/	54 (12 4)	25 (6 2)	325 (80 1)
vear	34 (13.4)	23 (0.2)	323 (80.4)

Table 3.	Distribution	of the	answers	that	students	gave to	the j	propositions	about	vitamin	D
		./				()					

* Vitamin D drug support is only administered intramuscularly	105 (26.0)	86 (21.3)	213 (52.7)
Vitamin D deficiency is common in infants, pregnant women and adolescents	299 (74.0)	20 (5.0)	85 (21.0)
Osteoporosis, fractures and muscle weakness can be seen in Vit D deficiency	369 (91.3)	12 (3.0)	23 (5.7)
Cancer incidence is higher in those with vitamin D deficiency	159 (39.4)	50 (12.4)	195 (48.3)
* Osteomalacia is a disease seen in children	203 (50.2)	66 (16.3)	135 (33.4)
Swellings of the chest are a kind of symptom of "O" leg rickets	268 (66.3)	17 (4.2)	119 (29.5)
* Rickets, a disease that occurred in adults	236 (58.4)	102 (25.2)	66 (16.3)
Rachitism (rickets) are common in children aged 2 months-3 years	112 (27.7)	13 (3.2)	279 (69.1)
Rickets is diagnosed by clinical findings, bone film, level of Ca- P-ALP	296 (73.3)	5 (1.2)	103 (25.5)
In adults, the blood 25 (OH) D level is at least 100 nmol / L	34 (8.4)	9 (2.2)	361 (89.4)
Toxic dose of vitamin D is 25,000 - 50,000 IU and over per day	34 (8.4)	17(4.2)	275 (68.1)
Since excess vitamin D can not be expelled from the body it is harmfull	244 (60.4)	63 (15.6)	97 (24.0)
* Taking more vitamin D can cause hypocalcemia	179 (44.3)	$\begin{array}{c} 8\overline{6} \\ (21.3) \end{array}$	139 (34.4)
Takling vitamin D increases the risk of kidney stones	220 (54.5)	36 (8.9)	148 (36.6)
* Taking more vitamin D will cause short stature in children	82 (20.3)	161 (39.9)	161 (39.9)

*: The correct answer to these questions are \wrong \choice

Discussion

Among students constituting the study group, the most accurately known question related to vitamin D was "the role of vitamin D on bone metabolism (91.6%)", compatible with a study in Australia indicating that most of respondents (76.0) reported that vitamin D is helpful on bone health (16). In this survey, only 2/3 of the students (62.9%) had a sufficient level of knowledge about vitamin D, in line with many studies; Vu et al., (2010) (16) reported that only 69% of office workers had a correct knowledge about vitamin D in a study conducted out related to knowledge and attitudes towards vitamin D and sun protection practices. In parallel with our study, in that study, it was determined that the most well-known benefit of vitamin D was on bone mineral health. Another study reported similar results; in a survey by Kung and Lee (2006) (17), only slightly more than half of the respondents had known the effect of vitamin D on bone health. These results show that the knowledge of vitamin D is insufficient in general. Thus, students or public should be informed or trained concerning D vitamin the importance of which is very important in every age group. While the level of knowledge about vitamin D in those studying at nursing department was the highest (67.6%), it was the lowest in those studying at management of health institutions (46.2%) (p<0.05), compatible with Kim et al., (2001)'s study (18). Actually, it is expected that knowledge level of students studying at administration of health institutions should be also sufficient. Because, as a manager at any health institution they must be aware of vitamin D. One reason for the low level of knowledge could be that lesson programs between 2 departments are different each other. Because due to the existence of health-related subjects such as physiology, pathophysiology and maintenance, level of knowledge of students at midwifery and nursing departments is better.

It was determined that the knowledge about the level of vitamin D was significantly higher in those in the last grades compared to the first grades (51.1% and 78.0%, respectively) (p<0.001), accordance with many studies (19,20). An explanation for this difference could be that they receive more lessons in health-related subjects.

The students' level of knowledge about vitamin D showed an increase as age increased. The level was only 46.5% 19 years old, whereas it was higher 22 years old (74.0%) (p<0.001). Similarly,

in the study by Lambrinou et al., (2009)(20), the level of knowledge showed increase with age. Hannon and Murphy (2007)(21) determined that level of knowledge showed increase as age increased in a study on students. These results suggest that the knowledge level of those in advanced classes is sufficient. In our study, sufficiency status of knowledge received previously about vitamin D was better in those who had a previous history of vitamin D compared to those without (p < 0.05), similar to many studies (22,23). In parallel, in this study, the level of knowledge in those with previous history of getting knowledge about vitamin D was higher when compared to those without (66.3% and 53.3%, respectively) (p<0.05). These findings show that the students obtained the knowledge about vitamin D sufficiently, indicating the importance of experience obtained previously. The source of knowledge in those with sufficient knowledge level was the lowest from written-verbal media (58.5%). However, there was no difference in terms of source of knowledge received about vitamin D between those whose level of knowledge was sufficient and those whose level of knowledge was insufficient (p>0.05). This result is not compatible with many studies indicating that media was in the first line among knowledge sources about vitamin D (16,17). According to this study results the fact that media was source of knowledge at the least rate preoccupies that mass media such as radio and television in our country is not used with right goals.

The question which was the most accurately known by the students was the phrase "Vitamin D is important in bone formation and growth" with a proportion of 91.6%, and the most wrongly known question was the phrase "the amount that an adult individual should take daily (96.3%)". In a similar study conducted in Australia on office workers, a big part of the participants reported that 'vitamin D is beneficial on bone health' (16). In parallel, Kung and Lee (2006)(17) have reported that most participants had known that 'vitamin D is beneficial to bone health'

In our study, the proportion of students whose level of knowledge about Vitamin D was sufficient was 62.9%. This result is similar to the result of many studies (24,25). Similarly, a study by Vu et al., (2010)(16) related to knowledge and attitudes concerning vitamin D and sun protection, 69% of office workers had a level of knowledge about D vitamin.

However, when results are generally evaluated, it suggests that the level of knowledge in terms of heath practices such as patient monitoring and giving education about the subject is not sufficient.

Conclusion

The low level of knowledge about vitamin D which is of great importance in maintaining health in every age group indicates that the importance of the issue during students' training is not highlighted enough. Therefore, training should be relevant to the issue at all stages of education.

Development of sensitivity and awareness of health professionals on public health-oriented subjects can be largely obtained in the student's period in which they received knowledge.

References

- 1. Zhang R, Naughton P: Vitamin D in health and disease: current perspectives. Nutr J 2010, 8:65-78.
- 2. Kull MJ, Kallikorm R, Tamm A, Lember M: Seasonal variance of 25-(OH) vitamin D in the general population of Estonia, a Northern European country. BMC Public Health 2009, 19:22-30.
- 3. Holick M: High prevalence of vitamin D inadequacy and implications for health. Mayo Clin Proc 2006, 81:353-373.
- Lee JM, Smith JR, Philipp BL, Chen TC, Mathieu J, Holick MF: Vitamin D deficiency in a healthy group of mothers and newborn infants. Clin Pediatrics (Phila) 2007, 46:42-44.
- 5. Hypponen EPC: Hypovitaminosis D in British adults at age 45 y: nationwide cohort study of dietary and lifestyle predictors. Am J Clin Nutr 2007, 85:860-868.
- 6. Winzenberg T, Powell S, Shaw KA, Jones G: Effects of vitamin D supplementation on bone density in healthy children: systematic review and meta-analysis. BMJ 2011, 342:267.
- Schierbeck LL, Jensen TS, Bang U, Jensen G, Køber L, Jensen JEB: Parathyroid hormone and vitamin D markers for cardiovascular and all cause mortality in heart failure. Eur J Heart Failure 2011, 13:626-632.

- 8. Visser M, Deeg DJ, Puts MT, Seidell JC, Lips P: Low serum concentrations of 25-hydroxyvitamin D in older persons and the risk of nursing home admission. Am Clin Nutr 2006, 84:616-622.
- 9. Bodnar L, Simhan H, Powers R, Cooperstein E, Roberts J: High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. J Nutr 2007, 137:447-452.
- 10. Holick M, Chen T: Vitamin D deficiency: a world wide problem with health consequences. Am J Clin Nutr 2008, 87:1080-1086.
- 11. Christie F, Mason L: Knowledge, attitude and practice regarding vitamin D deficiency among female students in Saudi Arabia: a qualitative exploration. Int J Rheum Dis 2011, 14:22-29.
- 12. Olmez D, Bober E, Büyükgebiz A, Cimrin D: The frequency of vitamin D insufficiency in health female adolescents. Acta Paediatr 2006, 95:1266-1269.
- 13. Özkan B, Doneray H, Karacan M, Vançelik S, Keskin Yıldırım Z, et al.: Prevalence of vitamin D deficiency rickets in the eastern part of Turkey. Eur J Pediatrics 2009, 168:95–100.
- 14. Hekimsoy Z, Dinc G, Kafesciler S, Onur E, Guvenc Y, Pala T, et al.: Vitamin D status among adults in the Aegean region of Turkey. BMC Public Health 2010, 23:782-787.
- 15. Ulitsky A, Ananthakrisknan SN, Naik A, Skaros S, Zadvornova Y, Binion DG, et al.: Vitamin D deficiency in patients with inflammatory bowel disease: Association with disease activity and quality of life. J Parenter Enteral Nutr 2011, 35:308-316.
- 16. Vu LH, van der Pols JC, Whiteman DC, Kimlin MG, Neale RE: Knowledge and attitudes about Vitamin D and impact on sun protection practices among urban office workers in Brisbane, Australia. Cancer Epidemiol Biomarkers Prev 2011, 19:1784-1789.
- 17. Kung AW, Lee KK: Knowledge of vitamin D and perceptions and attitudes towards sunlight among Chinese middle-aged and elderly women: A population survey in hong Kong. BMC Public Health 2006, 7:226-233.
- 18. Kim K, Kim M, Chung Y, Kim N: Knowledge and performance of the universal precautions by nursing and medical students in Korea. Am J Infec Cont 2001, 29:295-300.
- 19. Ziccardi S, Sedlak C, Doheny M: Knowledge and health beliefs of osteoporosis in college nursing students. Orthop Nurs 2004, 2:128-133.

- 20. Lambrinou E, Sourtzi P, Kalokerinou A, Lemonidou C: Attitudes and knowledge of the Greek nursing students towards older people. Nurse Educ Today 2009, 29:617-622.
- 21. Hannon C, Murphy KA: Survey of nurses' and midwives' knowledge of risks and lifestyle factors associated with osteoporosis. J Orthop Nurs 2007, 11:30-37.
- 22. Aksu A, Zinnuroglu M, Karaoglan B, Akin S, Kutsal Y, Atalay F, et al.: Research results of osteoporosis, education and awareness levels. Osteoporosis World 2005, 11:36-40.
- 23. Secginli S: Determination of knoweldge, belief and risk factors related to osteoporois. J Ataturk Uni School Nurs 2007, 10:77-88.
- 24. Berarducci A: Osteoporosis Education A health promotion mandate for nurses. Orthop Nurs 2004a, 23:118-120.
- 25. Berarducci A: Senior nursing students' knowledge of osteoporosis. Orthop Nurs 2004b, 23:121-127.

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Lipid therapy in cardiovascular drug poisoning

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Abstract

Verapamil and propranolol, drugs used in cardiovascular medicine, may cause death due to conduction abnormalities and drug refractory shock in high doses. Cardiotonic drugs are usually required for therapy. However, cardiogenic shock may deteriorate in spite of cardiotonic drugs in serious intoxications. Drug refractory cardiogenic shock had evolved in two separate patients admitted to our emergency department due to verapamil and propranolol intoxication. Vasopressor therapy failed in both patients and 100 mL of bolus 20 % lipid solution was given without delay followed by 1mL/kg infusion given in 1 hour. Shock findings resolved in both patients after lipid therapy. Application of intravenous lipid therapy (ILT) before generation of multi-organ dysfunction due to perfusion deterioration and usage of positive inotropic drugs may be reasonable. Also, its application before performing extracorporeal excretion methods is less invasive. Further prospective and controlled studies are required to determine the dosage and duration of ILT.

Keywords: Lipid therapy, emergency, poisoning.

Introduction

Intravenous lipid therapy (ILT) was firstly used in a patient suffering from local anesthetic toxicity and succeeded to save his life¹. Introduction of this therapy has led to generation of a new era in the treatment of poisoning due to lipophilic substances. In the following period, application of ILT in the treatment of poisoning due to lipophilic cardiovascular drugs such as verapamil and propranolol have come into question, which may lead to serious mortality and morbidity in toxic doses^{2,3}.

Verapamil and propranolol may lead to conduction abnormalities on electrocardiogram (ECG), drug refractory cardiogenic shock and cardiovascular death. Verapamil is a phenylalkylamine derived calcium channel blocker (CCB); whereas propranolol is a non-selective lipophilic beta-blocker, which also binds to proteins in high percentage. Both drugs lead to shock characterized by hypotension and bradycardia⁴. Cardiotonic drugs are usually required for therapy. However, in some cases with severe poisoning, cardiogenic shock doesn't resolve despite cardiotonic drugs. There are very few number of case reports in the medical literature demonstrating usefulness of ILT in the cardiotonic refractory severe poisoning due to these two drugs. Since prospective studies about this method are lacking, there is no consensus about timing and dosage of ILT in these circumstances.

In this paper, we've aimed to discuss the timing, administration and consequences of ILT in two separate patients whom admitted to our emergency department with signs of refractory cardiogenic shock due to verapamil and propranolol poisoning.

Case reports

Case 1

A 22 year-old female had been transferred to our clinic with a diagnosis of drug poisoning due to suicide intention. We've learned that she had admitted to our clinic after taking 1680 mg verapamil. In the physical examination performed at arrival she was conscious (Glasgow Coma Scale: 15), her blood pressure was 70/40 mmHg, heart sounds were rhythmic and normocardic with a pulse rate of 65/min and respiratory rate 20/min. No pathologic signs were seen on ECG. Her skin was pale and cold. Isotonic saline was initiated at a rate of 20 mL/kg. Gastrointestinal decontamination was performed and active charcoal was given. After 3000 mL of isotonic saline infusion, her blood pressure increased to 80/50 mmHg and she was transferred to the critical care unit. In the follow up period, her blood pressure decreased to 70/40 mmHg, pulse rate dropped down to 50/min

and she was anuric in spite of volume replacement therapy. Intravenous calcium gluconate (10 %; 50 mL) was given. Since she didn't respond to calcium gluconate therapy, intravenous bolus glucagone (0.1 mg/kg) was performed. Since patient was still unresponsive, intravenous dopamine was added to therapy. After vasopressor therapy, her hemodynamic status didn't improve and perfusion signs became worse. Since her clinical status was critically threatened despite vigorous therapeutic interventions, ILT was planned. One hundred and fifty mL of 20 % lipid solution was given in 5 minutes and her hemodynamic status improved immediately with a blood pressure of 95/55 mmHg and pulse rate of 65/min. To maintain the beneficial hemodynamic effects, 50 mL 20 % lipid solution was given in 1 hour and vasopressor therapy was sopped gradually. At the end of 1 hour, her blood pressure was 100/60 mmHg and pulse rate was 70/min. her hemodynamic signs remained stable after cessation of lipid solution. Her clinical and laboratory parameters were in normal range and she was discharged 48 hours after her hospitalization.

Case 2

A 21 year old female was transferred to our clinic with a diagnosis of drug intoxication due to suicide intention. She admitted to our clinic 4 hours after taking 960 mg of propranolol and 2250 mg of ferro-glycitine sulphate. Physical examination performed at arrival revealed that she was conscious (GCS: 15), hypotensive (90/60 mmHg), her heart sounds were rhythmic and bradycardic (48/ min) and respiratory rate was 18/min. there was sinus bradycardia on ECG. Her skin was pale and cold. Isotonic saline (20 ml/kg) infusion was initiated and simultaneous gastrointestinal decontamination and active charcoal ingestion was performed. Patient was transferred to critical care unit. In the follow up period, her blood pressure was 70/40 mmHg, pulse rate was 50/min and she was anuric in spite of volume replacement therapy. Intravenous bolus glucagon (0.1 mg/kg) was given. Since glucagon was unsuccessful, vasopressor therapy with dopamine was initiated. Patients' hemodynamic status didn't respond to dopamine either and her perfusion deteriorated. We decided to infuse ILT. After the bolus dose 150 mL given in 3-5 minutes, her blood pressure raised to 100/70 mmHg and pulse to 65/min. Vasopressor therapy was ceased gradually. In the follow-up, her blood pressure dropped down to 80/45 mmHg, and we decided to give another 50 mL of 20 % lipid solution. After the second dose, her blood pressure rised to 100/60 mmHg and her pulse rate was 64/min. Her hemodynamic status and laboratory tests remained stable and she was discharged 48 hours after her hospitalization.

Discussion

Intravenous lipid therapy was firstly performed as supportive therapy to standard resuscitation procedures in a patient with cardiac arrest due to local anesthetic intoxication and its success in turning the patient into life has led to generation of a new era in the treatment of lipophilic drug toxications¹. After this development, ILT was used in arrests due lipophilic drug toxicity⁵. The data and experiences about efficacy of ILT gained from animal studies and case reports have made physicians to gain clinical prediction about it. Although the controlled clinical studies done in human beings about this clinical situation are lacking, case reports seem to show significant importance in the generation of therapeutic algorithms in toxicology.

The first report about ILT in medical literature, is the presentation of a case report of a patient with cardiac arrest due to local anesthetic drugs. Rosenblatt an colleagues had used ILT during resuscitation of a patient with cardiac arrest after local anesthesia with bupivocain and the patient had turned back to life1. Local anesthetic drugs have both hydrophilic and lipophilic properties. Efficacy of the drugs increase in conjunction with the lipophilic property and penetration to cardiac and cerebral tissues increa se. At doses leading to toxicity, cardiovascular and central nervous system symptoms and signs dominate the clinical presentation^{6,7}. The toxicity of local anesthetic drugs is more profound in patients with carnitine deficiency. Local anesthetic toxicity in normal doses of local anesthesia had been found to be associated with carnitine deficiency and isovoluremic acidemia in a previous paper. Carnitine deficiency was thought to abolish local anesthetic drug metabolism which leads to occurrence of serious drug toxicity⁸. In the animal studies originating from these facts, ILT had been shown to cure poisoning signs in mice with local anesthetic drug toxicity.

The most feasible theory clarifying the mechanism of action of lipid solutions nowadays is sedimentation in lipid theory. Lipid molecules are stated to bind to lipophilic drugs and decrease their free amount by causing them to sediment in lipid emulsion⁹. When we review the medical literature, we can see ILT to be useful in the toxicity of lipophilic drugs such as verapamil, propranolol, diltiazem, ketiapin, bupropion, clomipramine and dosulepine^{2,3,10,11,12,13}.

Beta-adrenergic receptor blockers (BARB) and CCB's are widely used in the treatment of hypertension, coronary artery disease and arrhythmias. Toxications may be seen due to suicide intention or recurring dose intake in the elderly. The clinical properties of BARB's are dependent on the formulation of the molecule. This group of drugs highly binds to proteins and their distribution volume is very wide. Propranolol also highly binds to plasma proteins and is the most lipophilic BARB. Because of that, serious cardiotoxicity and central nervous signs are seen in its poisoning. Propranolol is more cardiotoxic than other BARB's, since it also blocks sodium channels¹⁴. Although the efficacies of CCB's are similar, their clinical application conditions are diverse. Since bradycardic effects of CCB's such as verapamil, a phenylalkylamine derived lipophilic CCB, appear in small excesses of daily doses; toxicity due to this substance is encountered frequently in emergency departments. The toxicity signs of propranolol and verapamil are quite similar. Both drugs lead to conduction abnormalities, hypotension and shock. Serious overdoses may cause refractory shock and cardiovascular collapse.

There are limited number of case reports and animal studies demonstrating the beneficial effects of ILT in cases with refractory shock who don't respond to conventional therapy procedures. In an animal study performed by Tebbut et al, lipid emulsion was given to rodents with verapamil toxicity just before cardiovascular arrest and dramatic improvements were observed¹⁵. In another animal study done by Bania et al, improvements in arterial blood pressure and heart rate were observed in the group of dogs treated with ILT added to standard medical treatment¹⁶.

The clinical outcome of refractory shock signs due to poisoning with 19.2 grams of verapamil in a 42 year-old patient had been narrated by Liang et al¹⁷. Transvenous temporary pacemaker was implanted for bradycardia. Intravenous calcium, vasopressors, euglycemic hyperinsulinemic therapy and venous hemodialysis had all been unsuccessful renal failure occurred due to hemodynamic failure. On the 3rd day of his hospitalization, ILT was added to therapy. Three hours after lipid infusion, patients' hemodynamic status improved and vasopressor doses were decreased¹⁷. Young and colleagues used ILT in a patient with refractory shock due to slow release verapamil poisoning². French et al reported a reduction in intravenous verapamil levels and improvement of toxicity signs after ILT application in a case with verapamil toxicity¹⁸.

In an animal study performed by Harvey et al, mean arterial pressure recovered better in the group of rabbits taking ILT for propranolol toxicity¹⁹. Jovic-Stosic and colleagues have reported a case of a patient with wide QRS tachycardia and shock after taking propranolol with alcohol. Only lipid solution was given for therapy and patient has recovered³.

Both of our patients had refractory shock after drug intake. Vasopressor therapy was given to them since they didn't respond to conventional therapy. However, their shock signs didn't improve and we have given ILT without delay. Hemodynamic status of both patients improved dramatically after ILT. Since shock signs has resolved, no more invasive procedures were required. Both patients were discharged 48 hours after their hospitalization.

Since lipid solutions are used for a long time as a part of parenteral nutrition, there are a lot of studies about their adverse effects, which are seen rarely in short term applications. Complications such as fat emboli, increased infection rates and thrombophlebitis are observed in patients receiving ILT for long terms and in excessive amounts as a part of parenteral nutrition. There are data demonstrating that the adverse effects are associated with the amount of ILT given for poisoning. When used in short terms, their adverse effect probabilities are less common then vasopressors. Plasma triglycerides may rise after application. Electrolyte disturbances, false elevations in hemoglobin and platelet counts are reported after lipid therapy²⁰. Amylase enzyme elevation without signs of pancreatitis was observed in a patient whom was given large amounts of ILT for drug poisoning²¹. Pnomatosis intestinalis was reported in a patient whom had taken 4200 mL of ILT for refractory verapamil intoxication in the third day of his hospitalization¹⁷. No adverse effects were observed in both of our patients.

Prospective placebo controlled studies are lacking about application of ILT for drug toxicity. However, lipid solutions are suggested in the guidelines of American Association of Anesthesiologists for patients with cardiac arrest due to local anesthetic toxicity. Following a bolus dose of 100 mL, 0.25 mL/kg of infusion (total dose not exceeding 3 ml/kg) is suggested⁵.

When we review the data derived from animal studies and case reports, ILT seems to be beneficial in propranolol and verapamil toxicity. However there isn't a consensus about application of this therapy since prospective studies are lacking about the dosage and duration of the therapy. We may suggest that ILT should be given to patients refractory to standard therapies without delay. Application of ILT before generation of multiorgan dysfunction due to perfusion deterioration and usage of positive inotropic drugs may be reasonable. Also, its application before performing extracorporeal excretion methods is less invasive. Further prospective and controlled studies are required to determine the dosage and duration of ILT.

References

- 1. Rosenblatt MA, Abel M, Fischer GW, Itzkovich CJ, Eisenkraft JB. Successful use of a 20% lipid emulsion to resuscitate a patient after a presumed bupivacaine-related cardiac arrest. Anesthesiology. 2006; 105: 217-8.
- Young AC, Velez LI, Kleinschmidt KC. Intravenous fat emulsion therapy for intentional sustainedrelease verapamil overdose. Resuscitation. 2009 May;80(5):591-3. Epub 2009 Mar 17.
- 3. Jovic-Stosic J, Gligic B, Putic V, Brajkovic G, Spasic R. Severe propranolol and ethanol overdose with wide complex tachycardia treated with intravenous lipid emulsion: a case report. Clin Toxicol 2011 Jun;49(5):426-30.

- 4. Salhanick S, Shannon M. Management of calcium channel antagonist overdose. Drug Saf. 2003; 26:65-79
- 5. http://www.lipidrescue.org
- 6. Cave G, Harvey M. Acad Emerg Med. 2009 Sep;16(9):815-24. Intravenous lipid emulsion as antidote beyond local anesthetic toxicity: a systematic review.
- 7. Weinberg GL. Current concepts in resuscitation of patients with local anesthetic cardiac toxicity. Reg Anesth Pain Med. 2002; 27: 568-75.
- 8. Weinberg GL, Palmer JW, VadeBoncouer TR, Zuechner MB, Edelman G, Hoppel CL. Bupivacaine inhibits acylcarnitine exchange in cardiac mitochondria. Anesthesiology. 2000; 92: 523-8.
- 9. Leskiw U, Weinberg GL. Lipid resuscitation for local anesthetic toxicity: is it really lifesaving? Curr Opin Anaesthesiol. 2009; 22: 667-71.
- Montiel V, Gougnard T, Hantson P. Diltiazem poisoning treated with hyperinsulinemic euglycemia therapy and intravenous lipid emulsion. Eur J Emerg Med. 2011 Apr;18(2):121-3.
- 11. Harvey M, Cave G. Intralipid outperforms sodium bicarbonate in a rabbit model of clomipramine toxicity. Ann Emerg Med. 2007; 49: 178-85.
- 12. Sirianni AJ, Osterhoudt KC, Calello DP, Muller AA, Waterhouse MR, Goodkin MB, Weinberg GL, Henretig FM. Use of lipid emulsion in the resuscitation of a patient with prolonged cardiovascular collapse after overdose of bupropion and lamotrigine. Ann Emerg Med. 2008; 51: 412-5.
- 13. Finn SD, Uncles DR, Willers J, Sable N. Early treatment of a quetiapine and sertraline overdose with Intralipid. Anaesthesia. 2009; 64: 191-4.
- 14. Elenhorn JM, Schonwald S, Ordog G, Wassenberger J. Elenhorn's medical toxicology: Chapter 32. Antiarrhytmic drugs. Beta blockers toxicity. 1143-46.
- 15. Tebbutt S, Harvey M, Nicholson T, Cave G. Intralipid prolongs survival in a rat model of verapamil toxicity. Acad Emerg Med. 2006; 13: 134-9.
- 16. Bania TC, Chu J, Perez E, Su M, Hahn IH. Hemodynamic effects of intravenous fat emulsion in an animal model of severe verapamil toxicity resuscitated with atropine, calcium, and saline. Acad Emerg Med. 2007; 14:105-11.
- 17. Liang CW, Diamond SJ, Hagg DS. Lipid rescue of massive verapamil overdose: a case report Med Case Reports. 2011 Aug 20;5(1):399.

- French D, Armenian P, Ruan W, Wong A, Drasner K, Olson KR, Wu AH. Serum verapamil concentrations before and after Intralipid® therapy during treatment of an overdose. Clin Toxicol (Phila). 2011 Apr;49(4):340-4. doi: 10.3109/15563650.2011.572556.
- 19. Harvey MG, Cave GR. Intralipid infusion ameliorates propranolol induced hypotension in rabbits. J Med Toxicol. 2008; 4: 71-6.
- 20. Smith NA. Possible side effects of Intralipid rescue therapy. Anaesthesia. 2010 Feb;65(2):210-1.
- 21. Marwick PC, Levin AI, Coetzee AR. Recurrence of cardiotoxicity after lipid rescue from bupivacaineinduced cardiac arrest. Anesth Analg. 2009;108: 1344-6.

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Depression among low vision patients

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Abstract

Background: Depression is common in people with visual impairment and has a number of debilitating effects on well-being and functioning. However, it is often not identified. The objective of this study was to explore depression in patients with visual impairment.

Methods: It was a causal-comparative study with convenient sampling (n=53) of patients with low vision problems that attend to the low vision clinic. After confirmation low vision diagnosis referred to psychiatric clinic for evaluation depression by Depression Anxiety Stress Scale (DASS-21). We compared five groups of low vision patients with 37 subjects of general population without problem as normal group.

Results: From the 53 low vision patients, 11 cases (21%) were Age-related Macular Degeneration (AMD), 7 cases (13%) cataract, 17 cases (32%) Retinitis Pigmentosa (RP), 7 cases (13%) Retinal Detachment (RD) and 11 cases (21%) Diabetic Retinopathy. Visually impaired patients had a higher rate of depression compared with people with normal vision. In Visually impaired groups, Diabetic Retinopathy and RP groups were depressed compared with people with normal vision.

Conclusion: Depression is a major concern in low vision patients and contributes more to functioning and well-being with on time treatments of depression in this group, they can look forward to a happier tomorrow.

Keywords: Depression, visual impairment, low vision.

Background

The National Eye Institute reports that low vision affects 3.3 million Americans 40 years and

older; this number is projected to reach 5.5 million by 2020 [1]. As the world population ages, the number of persons with major eye disease is increasing. Age-related macular degeneration (AMD) accounts for 54 percent of all blindness and is the leading cause of blindness among white Americans [1].Cataracts are the most prevalent eye disease in older persons and are the leading cause of blindness worldwide [2]. Almost all persons in their 90s will have had a cataract, and one half will have had cataract surgery [3]. Cataract surgery is the most common therapeutic surgical procedure reimbursed by Medicare with more than 1.5 million operations performed annually [2,3]. Diabetic Retinopathy is the leading cause of new blindness in adults 20 to 74 years of age [4].

Visual impairment is a severe reduction in vision that cannot be corrected with standard glasses or contact lenses and reduces a person's ability to function at certain or all tasks. The effect of visual impairment on visual function, health and wellbeing has been the subject of some previous studies [5].

Depression is common in people with vision impairment. Prevalence rate for depression among older vision impaired patients is from 25% to 45% compare to less than 20% (< 20%) among those with normal vision [6]. High rates of depression also have been found in visually impaired people in institutional setting [7]. The impact of depression in the visually impaired population is extremely debilitating and has been associated with increased difficulty in carrying out activities of daily living, mobility, and socializing [8]. Depression often goes undetected and untreated in patients with visual impairment, [8,9] and it may be difficult to distinguish chronic depression from a normal part of the grieving process associated with vision loss [10].

Depression increases incident of disability independent from sociodemographic factors, physical health status, cognitive functioning and vision-related limitations [11]. On the other hand, disability is a major risk factor for depression in low vision patients [12]. A variety of causes of depression were identified. Worse visual activity was believed to be related to depression. The significant life changes experienced by a person with vision loss such as loss of independence, and increased difficulty under taking activities of daily living and hobbies, as well as emotional reactions to vision loss such as guilt, feeling of worthlessness, and loss of control were also frequency cited as important factors contributing to depression.

Therefore, Physical disability and depressive symptoms when present together can initiate a spiraling decline in the physical and psychological health and quality of life. The purpose of this study was to explore depression in Iranian low vision population.

Methods

It was a causal-comparative study with convenient sampling of patients with vision impairment that attending the low vision clinic. From two academic center of low vision, one of them randomly selected. (Low vision clinic of Shahid Beheshti University of medical science).

This study was done for 6 months period. All of the patient refered by ophthalmologist with low vision diagnosis, after evaluation by optometrist they entered to study. Also they had no diagnosis of depression in past psychiatry history.

The protocol for this study was approved by the ethics review board of the institute. Written informed consent was obtained from all participants. The statistical population of the research consists of 53 low vision patients and 37 normal cases were subjected to evaluation for depression by depression subscale of Depression Anxiety Stress Scale (DASS-21).

DASS-21 is a self-report measure. The depression subscale consists seven items which largely assess dysphoria, anhedonia, hopelessness, devaluation of life and inertia [13]. The seven items of depression subscale refer to the past week. Each item rated on a 4 point Likert scale from 0 which means "did not apply to me at all" to 3 "applied to me very much or most of the time". For depression scale, the score can range from 0 to 21.

The DASS-21 demonstrated positive psychometric properties among Iranian population. Results of our previous study indicate that the DASS-21 has overall good-to-excellent internal consistency, good stability over time, a three factor structure consistent with other findings among mostly English speaking population, very good convergent validity and acceptable discriminate validity especially with respect to the depression scale. The DASS-21 psychometric strengths provide support for its use in both clinical and research setting in the Iranians population[14].

Data analysis

Analysis was carried out using SPSS 16.0 for windows (SPSS Inc, Chicago, IL USA). Descriptive analyses were computed in terms of mean and standard deviation for the entire sample as well as for group comparison between low vision and normal population. Low vision patients were compared with normal group on various sociodemographic using independent-sample T-test for parametric variable, and using Chi-square test for non-parametric variables. For comparison between two group low vision and normal population on depression variable using independent- sample T-test. For comparison between any five group low vision including Age-related Macular Degeneration (AMD), Cataract, Retinitis Pigmentosa (RP), Diabetic Retinopathy and normal population on depression variable using analysis of Covariance (ANCOVA).

Results

From 53 low vision subjects with mean age of 45.92 ± 21.99 , 18 cases (34%) were macular degeneration (that including AMD (11 cases, 21%) and Cataract (7 cases, 13%)), 17 cases were RP (32%), 7 cases RD (13%), and 11 cases diabetic (32%). The sociodemographic characteristics of the sample are displayed in table 1. There were 15 (42%) male and 21 (58%) female in the normal group and 34 (64%) male and 19 (36%) female in the low vision patients. The low vision and normal groups did not differ statistically significant on de-

mographic parameters (gender, education and Marital status) but differed significantly on age variable (P< 0.05). The comparisons among six groups of this study (AMD, Cataract, RP, RD, Diabetic, normal) in mean age show statistically significant difference (F = 21.24, P = 0.0001). Also, the results of analysis with post-hoc test show that only there is statistically significant difference in mean age of AMD group compared with others (P< 0.05).Therefore, in this study age variable have been controlled as covariate variable.

Table 2 shows means and standard deviations for depression scale of DASS-21 in the sample groups. The mean depressive score in low vision patients and normal group was 8.45 ± 5.4 , and 4.38 ± 3.34 respectively. Findings of t-test for comparison between low vision and normal score on depression variable indicated that statistically significant difference are existed in low vision patients with normal group (T value = 4.06, df = 88, P= 0.0001). The results of ANOCVA tests for comparison between six groups (with age control) show that significant difference on depression variable are existed (F = 8.19, P=0.001). The pair wise comparisons (Post Hoc) Tukey HSD between six groups indicated that there were statistically significant difference in mean depression score only between diabetic and RP groups with normal group, and mean depression score in normal group is lower than two groups. The diabetic and RP groups in comparison with Cataract are significantly depressed. (Table3).

The above findings show that higher rate of depression in diabetic and RP groups were seen in comparison with normal and Cataract groups.

Discussion

The effect of low vision on well-being, with its frequency and economic effect, makes visual impairment an important public health issue, even

Variables	Low vision Group (n=53)	Normal Group (n=37)	Total sample (n=90)	P value
	Ι			
Age	45.92 ±21.99	36.68 ±12.12	42.08 ± 19.07	0.02*
Gender	Count (%)	Count (%)	Count (%)	
Male	34 (%64)	15 (%42)	49 (%55)	0.061
Female	19 (%36)	21 (%58)	41 (%45)	
Marital status				
Never married	15 (%29)	14 (%28)	29 (%33)	0.49
Married	38 (%71)	23 (%62)	50 (%67)	
Education				
High School diploma	26 (%50)	10 (%27)	36 (%40)	0.06
Bachelor and Over	26 (%50)	37 (%73)	54 (%60)	
Sig* < 0.05				

Table 1. Sociodemographic characteristics of the sample

Table 2. Means and standard deviations for depression scale of DASS-21 in the sample groups

Group	N number	Variables		
		Depression	Age	
		Mean± SD	Mean± SD	
Low vision	53	8.45 ± 5.40	$45.92 \pm 21.99)$	
A.M.D	11	7.91 ± 6.06	78.36 ± 7.28	
Cataract	7	3.00 ± 2.16	35.86 ± 14.08	
R.P	17	9.59 ± 5.22	31.76 ± 11.48	
R.D	7	6.57 ± 4.79	40.57 ± 16.99	
diabetic	11	11.91 ± 3.96	45.09 ± 17.87	
Normal	37	4.38 ± 3.39	36.68 ± 12.12	

1	1	5		
Group Diagnosis		Mean Difference	Std. Error	sig
Normal	A.M.D	-3.53	1.47	0.17
	cataract	1.37	1.76	0.97
	R.P	-5.21	1.25	0.001**
	diabetic	-7.52	1.47	0.0001**
	R.D	-2.19	1.77	0.82
A.M.D	cataract	4.91	2.07	0.18
	R.P	-1.68	1.66	0.92
	diabetic	-4.00	1.82	0.25
	R.D	1.34	2.07	0.99
R.P	cataract	6.59	1.92	0.01**
	diabetic	-2.32	1.65	0.73
	R.D	3.01	1.92	0.62
Diabetic	cataract	8.91	2.07	0.001**
	R.D	5.33	2.07	0.11
R.D	cataract	3.57	2.21	0.63
Sig** < 0.01 Sig* < 0.05				

Table 3. Pair wise comparisons (POST HOC) Tukey HSD

in developed countries. The results of this study showed a higher rate of depression in low vision patients that is similar with other studies [6,7]. The De Leo and several other studies identified an increased risk of suicide in people with visual impairment [15,16,17,18] it seems that visual impairment has a negative impact on social relationships and well-being.

In our study, we found evidence that the cause of visual loss had an impact on depression. Depression in Diabetic Retinopathy and Retinitis Pigmentosa patients were higher than normal and Cataract groups. We believed a range of factors were associated with depression including the degree of vision loss, patients understanding of their condition, personal characteristics and coping styles, however other studies suggest that degree of vision loss is not the main predictor of depression on people with vision impairment. Instead, visual function, comorbidities and coping strategies have been identified as stronger predictors in this group [9,19,20], With exclusion comorbidities in our study other factors were prominent.

Conclusions

Visually impaired patients have a higher rate of depression compared with normal population. Effective medicinal and therapeutic regimens are also available to treat depression, and treatment is most effective when implemented early. Despite this, depression often remains undetected and there for untreated in people with vision loss. The point of this study is that depression in low vision patients need to recognize. With early treatments of depression a growing proportion of these patients can enjoy the best quality of life.

List of abbreviations:

Age-related Macular Degeneration (AMD) Retinitis Pigmentosa (RP) Retinal Detachment (RD) Depression Anxiety Stress Scale (DASS-21) Analysis of Covariance (ANCOVA)

Authors' contributions

Dibajni P and Moghadasin M : Developed the initial idea and design of the study.

All authors revised and commented on drafts of the manuscript.

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References

 National Eye Institute. Vision loss from eye diseases will increase as Americans age [press release]. April 12, 2004. http://www.nei.nih.gov/news/pressreleases/041204. asp. Accessed August 3, 2007.

- 2. Solomon R, Donnenfeld ED. Recent advances and future frontiers in treating age-related cataracts [published correction appears in JAMA. 2003;290(22):2943]. JAMA. 2003;290(2):248-251.
- 3. Taylor HR. Fred Hollows lecture. Eye care for the community. Clin Experiment Ophthalmol. 2002;30(3):151-154.
- 4. The Eye Diseases Prevalence Research Group. Statistics and data. Citations and abstracts from April 2004 Archives of Ophthalmology. Causes and prevalence of visual impairment among adults in the United States. http://www.nei.
- 5. Chia EM, Mitchell P, Ojaimi E, Rochtchina E, Wang JJ. Assessment of vision related quality of life in an older population sub sample . The Blue Mountains Eye Study. Ophthalmic Epidemiol 2006;13:371-377.
- 6. Evans JR, Fletcher AE, Wormald RP. Depression and anxiety in visually impaired older people. Ophthalmology 2007; 114: 283-8.
- 7. IP SP,Leung YF,Mak WP.Depression in institutionalised older people with impaired vision.Int J Geriatr Psychiatry 2000;15:1120-4.
- 8. Horowitz A, Reinhardt JP. Adequacy of the mental health system in meeting the needs of adults who are visually impaired. J Vis Impair Blind 2006; 100: 871-4.
- Rovner BW, Casten RJ, Hegel MT, Leiby BE, Tasman WS. Preventing depression in age-related macular degeneration. Arch Gen Psychiatry 2007;64: 886-92.
- 10. Egede LE. Failure to recognize depression in primary care: issues and challenges. J Gen Intern Med 2007; 22:701-3.
- 11. Shmuely-Dulitzkiy, Rovner BW, Zisselman P. Impact of depression on functioning in elderly patients with low vision .Am J Geriatr Psychiatry ,1995; 3:325-9.
- 12. Cole MG, Dendukuri N. Risk factors for depression among elderly community subjects: Asystematic review and meta-analysis. Am J Psychiatry 2003; 160:1147-56.
- 13. Lovibond PF& Lovibond SH. The structure of negative emotional states: Comparison of the depression anxiety stress scales (DASS) with the beck depression and anxiety inventories. Behavior and Research and Therapy, 1995a; 33, 335-345.
- 14. Asghari A., Saed F., Dibajnia P. Psychometric properties of the Depression Anxiety Stress Scale-21 (DASS-21) in a non-clinical Iranian sample. International Journal of psychology.2008;2: 82-102.
- 15. De Leo, D., Hickey, P.A., Meneghel, G. and Cantor, C.H. (1999) Blindness, fear of sight loss, and suicide, Psychosomatics, 40, 339-344.

- Lam, B., Christ, S., Lee, D., Zheng, D. and Arheart, K. (2008) Reported visual impairment and risk of suicide: the 1986-1996 national health interview surveys, Archives of Ophthalmology, 126, 7, 975-80.
- 17. Mitchell, J. and Bradley, C. (2006) Quality of life in age-related macular degeneration: a review of the literature, Health and Quality of Life Outcomes, 4, 1, 97.
- Waern, M., Rubenowitz, E., Runeson, B., Skoog, I., Wilhelmson, K. and Allebeck, P. (2002) Burden of illness and suicide in elderly people: case-control study, BMJ, 324, 7350, 1355.
- 19. Rovner BW,Casten RJ. Activity loss and depression in age related macular degeneration. Am J Geriatr Psychiatry 2002; 10:305-10.
- 20. Dreer LE, Elliott TR, Berry J, Fletcher DC, Swanson M, Christopher MC Neal J.Cognitive appraisals, distress and disability among persons in low vision rehabilitation. Br J Health psychol 2008;13:449-61.

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Does Ramadan fasting influence breastmilk?

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Abstract

Objective: Ramadan is the holiest month of Islam and muslims fast during this month, however, pregnant and nursing women are not obliged to fast. They can postpone this religious duty to a further time. Despite this flexibility a great number of nursing and pregnant women fast. The aim of this study is to determine the impact of maternal Ramadan fasting on breast-fed infants and to discuss the beliefs of mothers about fasting during nursing.

Material and method: Between 10th and 20th days of Ramadan, a total of 119 mothers of babies between 1.5 and 12 months old were included in our study.

Results: The mean age of 119 participated babies (male/female:63/56) was 5.5±2.9 months. Sixty five (54,6 %) mothers were fasting. While the education level and the exclusively breastfeeding ratio of fasting mothers were significantly lower than non-fasting mothers, their babies were significantly older. When fasting, all nursing mothers (38 of total) drank, ate and rested more than their regular habits in order not to decrease the breast milk rate. While half of fasting mothers did not perceive decrease in breast milk, 25% of mothers decreased supplementing time and frequency of breastfeeding. Percentage 74 of mothers thought that while fasting breast milk decreases, 67% of mothers thought that nursing mothers should not fast when the duration between sunrise and sunset is longer and 80,5% of mothers thought that nursing mothers should not fast.

Conclusion: Child care practitioners should know more about fasting and should educate mothers especially the ones with low level of education about the importance of breastfeeding. In this Islamic duty which closely related to infants health, pediatricians should be able to develop empathy, and convince the nursing mothers to postpone fasting.

Keywords: Ramadan fasting, breastfeeding, attitudes of mothers.

Introduction

Ramadan, the ninth Arabic lunar month, is the holiest month of Islam. Throughout this month, Muslims refrain from eating, drinking, smoking and having sexual contact from sunrise to sunset. Every year, Ramadan takes precedence for 10 days, so depending on the season the duration of the daily fasting varies from 11 to 18 hours, annually. In summer term, the fast is longer than winter term. According to Islamic tradition, sick patients, travelers, pregnant, nursing and menstruating women are not obliged to fast during Ramadan (1). They will fast at an acceptable date for them later. Despite this flexibility a great number of nursing and pregnant women fast around the world.

It is well known that breastfeeding is very important for infants and is associated with their biological, psychological and intellectual development. The aim of this study is to determine the impact of maternal Ramadan fasting on breast-fed infants and to discuss the beliefs of mothers about fasting during nursing.

Material and methods

Between 10th and 20th days of Ramadan in September 2008, a total of 119 (male/female:63/56, mean age:5.5±2.9 months) infants' mothers who attended to pediatric clinics at Dr. Sami Ulus Gynecology and Child Health and Health Education and Research Hospital were enrolled.

Informed consent was taken from the mothers participating in the study. The questionnaire included close-ended and yes-no questions. One pediatrician from Sami Ulus Hospital asked these questions to the participant mothers. SPSS-PC 16.0, including Chi-Square Test, was used for statistical
analyses. Statistical significance was defined as P value less than 0,05.

Results

One hundred and nineteen infants participated to this study. Fifty six (47,1 %) infants were girls versus 63 (52,9 %) were boys.

Sixty five (54,6 %) mothers were fasting. Fifty four infants were between 1,5 months and 6 months vs 65 infants were older than 6 months. Forty two percentage of mothers of infants smaller than 6 months were fasting, with respect to 76,7 % mothers of infants older than 6 months (p<0.001). The clinical, anthropometric characteristics of fasting and nonfasting mothers and their infants are reported in Table 1.

Sociodemographic characteristics of fasting and nonfasting mothers are documented in Table 2. Although 56 (62,9%) of primary school graduates were fasting, only 9 of high school or university graduates (30%) were fasting (p<0,05).* Forty two (36 %) infants were exclusively breastfeeding, 45 (38 %) were breastfeeding in combination with supplements, 32 (27%) were taking only supplemental foods. Seventy one percentage of nursing mothers were not fasting whereas 38 % of mothers nursing and giving supplements were fasting (p<0.001). We asked questions to 46 nursing and fasting mothers (38% of total).When fasting, all of them drank, ate and rested more than their regular habits in order not to decrease the breast milk rate. Twelve (26,2%) of these women decreased supplementing time and frequency of breastfeeding (p<0.05). Twenty (43,5%) of mothers did not perceive decrease in breast milk (p>0.05).

We asked questions about mothers' beliefs about breastfeeding and fasting. The choices of answers were 'yes', 'no' or 'no idea' (Table 4).Eighty eight (74%) of mothers thought that breast milk decreases while fasting, however, half of them were fasting.

Table 1. Clinical, anthropometric characteristics in fasting and nonfasting mothers and their infants (mean \pm SD)

	Fasting mothers (n=65)	nonfasting mothers (n=54)	P – value
Age (years)	26.1±6.2	24.9 ± 6.9	0.23
Body Mass Index (kg/m ²)	25 ±4.8	23.8 ±3.3	0.1
Infant age(months)	6.6 ±2.7	4.1 ±2.6	0.001
Infant weight (kilograms)	7.3 ±1.5	6.3 ±1.4	0.001
Infant height (centimetres)	65.9 ±5.2	62.6 ±6.3	0.002
Birth weight (kilograms)	2.9±0.5	3±0.6	0.3

Table 2. Sociodemographic characteristics of fasting and nonfasting mothers

Variables	Percentage of fasting(%)	Percentage of non fasting(%)	P-value
Graduating primary school and under	62.9	37.1	0,045
Graduating from high school and upper	30	70	0.003
Working	35.7	64.3	0.1
Living in an extended family	51.9	48.1	0.5
Having single child	52	48	0.2
Breastfeeding	28.6	71.4	0.000
Giving supplements	62.2	37.8	0.000
Infant age 6 months and under	42.1	57.9	0.000
Infant age older than 6 months	33	10	0.000

We asked 'Should nursing mothers fast?'

Eighty (67%) of them thought negatively, but 34 (42%) of these mothers were fasting and 25(21%) of them thought positively and 20(80%) were fasting.

The exact dates of *Ramadan change* every *year*. In 2008, fasting time was approximately 14 hours. We asked mothers 'Does the duration of fasting time influence the fasting?' When the days are longer, 95(80,5%) of total thought that nursingmothers should not fast and 46 (48,4%) of these were fasting.

Discussion

Ramadan fasting is very important for Muslims all around the world. Pregnant and nursing women should not fast for not to harm the life and health of offspring. These women should fast later in an appropriate time (1). The American Academy of Pediatrics (AAP) recommends exclusive breastfeeding for a minimum of 4 months and preferably for six months (2). Breastfeeding is very important for infants' biological, psychological and intellectual development. Contact between mother and child during breast feeding may facilitate the bonding process and enhance development (3). Lactating women have higher circulating levels of prolactin and oxytocin and these hormones trigger feelings of calmness, well-being, nurturing behavior and may facilitate positive mother-child interactions which may in turn facilitate neurodevelopment (4). According to TDHS-2008 (Turkey Demographic and Health Survey) breastfeeding is common in Turkey and ninety seven percentage

of all children are breastfed for certain period of time, but onset of giving supplements is as early as 2 mo. Forty two percentage of children smaller than 6 mo are exclusively breast-fed. After the sixth month, feeding with other types of milk and complementary foods is more common than breastfeeding (5). Like THDS outcomes, our patients had exclusively breastfed for average of only 3.5 months (0-12 months).

Our hospital is in a rural area in the second big city and the capital city of Turkey, Ankara. The socioeconomic level of our patients is not high. Seventy five percentage of mothers had graduated from primary school. Compatible with the literature when education level is high, fasting ratio is low (6). We think that the importance of breastfeeding is well known by highly educated mothers. The average age of non-fasting mothers' infants was 4,1 mo and fasting mothers' was 6,6 mo. Fasting mothers' infants are older and take supplements so their weights and heights are bigger.

There is no significant difference among the age of mothers, body-mass index, family type, the number of offspring and having a job. Contrary to the literature, BMI and family type do not effect fasting (7). In the literature no significant loss of body mass in adults pointed to fasting. Body mass loss may also be attributed to a decrease of glycogen-bound water stores, extracellular volume contraction secondary to a lower sodium intake, and a moderate degree of hypohydration with little loss of body tissue (8-13).

When we asked some subjective questions to mothers (46) who were fasting and nursing, all of them had precautions such as drinking, eating

Questions	Yes (%)	No(%)	p-value
Did breastfeeding ratio decrease?	26.1	73.9	< 0.05
Did you feel decreasing of your breastmilk?	56.5	43.5	>0.05
Did you increase supplementing food?	60.5	39.5	< 0.05

Table 3. Objective and subjective attitudes of fasting and nursing mothers

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		Fasting			Nonfastir	ng
Questions	N Z (0/)	NI (0/)	No idea	Yes	NI (0/)	No idea
	Yes (%)	NO(%)	(%)	(%)	NO(%)	(%)
When fasting breastmilk decreases	67.7	24.6	7.7	81.5	11.1	7.4
Nursing mother should fast	30.8	52.3	16.9	85.2	9.3	5.5
If fasting interval is short nursing mother should fast	80	13.8	6.2	50.0	48.1	1.9

Table 4. Beliefs of fasting and nonfasting mothers

and resting more for not decreasing their breast milk. Food and fluid intake are mainly nocturnal and usually food frequency and quantity, sleep duration at night and daily physical activity are reduced. There is a tendency to consume foods and drinks that are richer in carbohydrates than those consumed during other months of the year. As our fasting and nursing mothers Rakicioglu et al (14) found that lactating women consumed more fluid with sugar to increase the secretion of their milk. Water deprivation is functionally characterized by maximum urine concentration. Shirrefs et al (15) found that over the morning and afternoon collection periods, urine volume, sodium, potassium and total solute excretion were lower, and urinary osmolality was higher during Ramadan than either before or after Ramadan. During Ramadan, the osmolality of the urine samples collected in the afternoon were very high, indicating effective water conservation (15). Rakicioglu et al aimed to determine the effect of Ramadan fasting on maternal nutrition and breastmilk composition. They found that Ramadan fasting had no significant effect on the macronutrient composition of the breast milk and consequently the growth of the infants(14). Also Bener et al concluded that Ramadan fasting did not affect breast milk quality(16).

Twenty six percentage of these women decreased nursing frequency, but 74 % did not(p<0.05). There is a risk factor for discontinuing breastmilk in these 12 infants. More than half of fasting women perceived decrease in breast milk and only 4 of them braked the fast. Twenty three (60.5%) of 38 fasting mothers who gave supplements in combination with breastfeeding increased food supplementation.

We intended to learn mothers' beliefs and compare their beliefs with their practices. Eighty eight (74%) of mothers thought that breastmilk decreases when women are fasting but half of them were fasting. Twenty two (18,5%) thought it doesn't decrease and 6 (27,3%) of these weren't fasting. When the fasting duration is long most of the mothers (%80,5) thought nursing mothers shouldn't fast but half of them(48.4%) were fasting. Our patients beliefs and practises about breastfeeding and fasting were conflicting. We should not exclude the effects of traditional beliefs and society on their practices. Mothers whose offsprings were young and exclusively breastfeeding did not fast. We think in our country the awareness of importance of breastfeeding is high enough, but giving supplements before 6 mo is frequent. Child care practitioners should know more about fasting and should educate especially less educated mothers about the importance of breastmilk. They should develop empathy and should not command about this religious subject.

References

- 1. Mutlu. I. Fasting. Big book explaining the principles of Islam. (1th ed). Mutlu I. Istanbul, Mutlu Co, 1995; 461-501.
- 2. Stettler N, Bhatia J, Parish A et al.Nutrition. Nelson Textbook of Pediatrics (18 th ed). Behrman ER, Kliegman RM, Jenson HB (eds): Philadelphia, WB Saunders Co, Elsevier Science, 2007; 160-165.
- 3. Stuard Macadam P. Biocultural perspectives. In: Breastfeeding:Biocultural perspectives. Editors: Stuard Macadam P,Dettwyler KA. New York: Aldine de Gruyter, 1995.
- 4. Drane DL, Logemann J.A. A critical evaluation of the evidence on the association between type of infant feeding and cognitive development. Paediatric and Perinatal Epidemiology, 2000; 14(4): 349–356.
- 5. TDHS-2008 (Turkey Demographic and Health Survey).
- 6. Ozturk Ertem I, G Kaynak, C Kaynak et al. Attitudes and practices of breastfeeding mothers regarding fasting in Ramadan. Child Care Health Dev.2001;27:545-54.
- 7. Zohreh Kavehmanesh, Hassan Abolghasemi. Maternal Ramadan Fasting and Neonatal Health. Journal of Perinatology. 2004; 24:748–750.
- 8. JB Leiper, AM Molla. Effects on health of fluid restriction during fasting in Ramadan. European J of Clinical Nutrition. 2003; 57:30-38.
- 9. Afifi ZE et al. Daily practices, study performance and health during the Ramadan fast. J. R. Soc. Health.1997;117: 231–235.
- 10. Laajam MA et al. Ramadan fasting and non-insulin-dependent diabetes: effect on metabolic control. East Afr. Med. J. 1990;67:732–736.
- 11. El Ati J, Beji C & Danguir J et al.Increased fat oxidation during Ramadan fasting in healthy women: an adaptive mechanism for body-weight maintenance. Am. J. Clin. Nutr. 1995;62:302–307.

- 12. Ramadan J, Telahoun G, Al-Zaid NS et al. Response to exercise, fluid, and energy balances during Ramadan in sedentary and active males. Nutrition. 1999;15:735–739.
- 13. Frost G & Pirani S et al. Meal frequency and nutritional intake during Ramadan: a pilot study. Hum. Nutr. Appl. Nutr. 1987;41:47–50.
- Rakıcıoglu N, Samur G, Topcu A et al. The effect of Ramadan on maternal nutrition and composition of breastmilk. Pediatrics International. 2006;48:278-83.
- 15. Shirreffs SM. Markers of hydration status. Eur. J. Clin. Nutr:2003;57:6-9.
- 16. Bener A, Galadari S, Gillet M et al. Fasting during the holy month of Ramadan does not change the composition of breast milk.Nutrition Research. 2001;21:859–864.

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Minimal invasive bronchoscopic therapy of tracheal mucosa-associated lymphoid tissue lymphoma: two cases report and literature review

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Abstract

Mucosa-associated lymphoid tissue (MALT) lymphoma derived from tracheobronchial mucosa is an extremely rare disease and no standard treatments have been established. Considering its tendency of maintaining localized for a long period, bronchoscopic minimal invasive procedure for diagnosis and treatment may have some advantages over other major operations. We reported two patients of bronchial MALT lymphoma, who were in emergencies due to the obstructing masses in the bronchus. Both patients were successfully treated with a combined regime of bronchoscopic resection of intraluminal occupying lesions and systemic chemotherapy. These cases emphasized a critical role that bronchoscopic therapy played in the management of MALT lymphoma derived from central airway.

Key words: Mucosa-associated lymphoid tissue lymphoma, (MALT), bronchoscopic minimal invasive procedure

Introduction

Mucosa-associated lymphoid tissue (MALT) lymphoma, a distinctive subgroup of low grade malignant B cell extranodal non-Hodgkin's lymphoma, is derived from lymphoid tissues located under the epithelia of gastrointestinal, respiratory and urogenital tracts [1]. Primary MALT lymphoma from the tracheobronchial mucosa is an extremely rare disease [2, 3]. Increasing evidence has suggested that the development of bronchial MALT lymphoma may be associated with chronic inflammatory response to bacterial, viral and other stimulants [2-6]. Generally, the disease has a favorable response to different kinds of therapeutic modalities and has an estimated 10-year survival rate of 72% [7]. However, because of its rarity, so far there is no standard therapeutic method for the disease. For those patients who can't bear a major surgery or who have diffused and obstructive intraluminal lesions, minimal invasive therapy by bronchoscopy might provide a better therapeutic choice. Here we reported two patients, whose trachea and bronchia were nearly obstructed by MALT lymphoma, were successfully treated with bronchoscopic therapy combined with subsequently systemic chemotherapy.

Case report

Case 1: A 55-year-old male patient was admitted to our hospital on December 2010 with complaints about progressive cough and dyspnea for eight months. Physical examination revealed scattered wheezing and moist rales in the chest. He had cyanosis and his breath rate was 24 times per minute. His Karnofsky performance status score was 40 and dyspnea index was 4. Chest computerized tomography (CT) showed a narrowed trachea suppressed by a mass (Fig.1a). The bronchoscopic inspection showed an irregular mucosa intrusion at the mid-segment of the trachea (Fig.1b). After a biopsy was made, argon plasma coagulation (APC) and carbon dioxide cryotherapy under bronchoscopy were conducted to completely eliminate the mass and recanalize the trachea (Fig.1c). His dyspnea was immediately relieved. The dyspnea index returned to 0 and Karnofsky performance status score returned to 90. The biopsy established a diagnosis of MALT lymphoma (Fig.1d). Immunohistochemical analysis revealed Vim (+), LCA (+), CD20 (+), CD3 (-), CD56 (-), CD99 (-), CK (-), Syn (-). No significant abnormalities were found in a whole body Positron Emission Tomography (PET/CT) scan or in a bone marrow aspirating biopsy. The patient had undergone bronchoscopic APC and carbon dioxide cryotherapy for three times since December 2010. Simultaneously, he had six sequential cycles of systemic chemotherapy with R-CHOP regime (Rituximab 700mg d1+ Cyclophosphamide 1400mg d2+ Epirubicin 110mg d2+ Vincristine 2mg d2+ Prednisone 100mg d2-6). The repeated chest CT and bronchoscopy inspection demonstrated disappearance of the lesion (Fig.1e) and smooth tracheal lumen (Fig.1 f). He was in a stable situation for more than one year without any evidence of recurrence by repeated PET/CT scans.

Case 2: A 61-year-old female patient was admitted to our hospital, complaining about cough for six months and aggressive short of breath for two weeks. On physical examination, her Karnofsky performance status score was 80 and dyspnea index was 3. There were no palpable lymph nodes in her neck. Her collapsed left chest was noted and no breath sound could be heard. The chest three-dimension reconstruction CT revealed left lung atelectasis and some masses obstructing the trachea and the left main bronchus (Fig.2 a). No enlarged

lymph nodes in mediastinum were detected by CT. Bronchoscopy inspection manifested that the tracheal mucosa was covered with diffused pavestone-like lesions and the right main bronchus was completely blocked by a mass (Fig.2b). APC and carbon dioxide cryotherapy were performed, resulting in recanalization of the left main bronchus (Fig.2c) and immediate alleviation of dyspnea. The biopsy demonstrated B cell non-Hodgkin's lymphoma with immunohistochemistry CD20 (+), CD56 (partially+) (Fig.2 d). No enlarged lymph nodes in neck, abdomen and pelvis were detected by a whole body CT scan. No abnormalities were found in a bone marrow aspiration. From July 2011, the patient had finished four sequential cycles of systemic chemotherapy with CHOP regime (Cyclophosphamide 1100mg d1+ Epirubicin 100mg d1+ Vincristine 2mg d1+ Prednisone 100mg d1-5). The repeated chest three-dimension reconstruction CT showed disappearance of the lesions and normal left main bronchus (Fig.2e). The patient achieved a complete remission and currently remained free of symptoms.



Figure 1. Bronchoscopic, chest CT and pathology images in case 1 with bronchial mucosa-associated lymphoid tissue (MALT) lymphoma.

(a) The chest CT showed a mass in the trachea and the lumen was narrowed. (b) Bronchoscopy showed thickening and irregular intrusion of mucosa in the middle segmental of the trachea. (c) After bronchoscopic argon plasma coagulation (APC) and carbon dioxide cryotherapy, the mass was almost eliminated and the lumen was dilated. (d) Biopsy pathology showed MALT lymphoma (magnification 10×20). (e) One month later, the repeated chest CT demonstrated the size of lesion decreased. (f) The repeated bronchoscopy showed a clear lumen in the trachea



Figure 2. Bronchoscopic, chest CT and pathology images in case 2 with bronchial mucosa-associated lymphoid tissue (MALT) lymphoma.

(a) Three-dimension reconstruction chest CT demonstrated left lung atelectasis. Soft tissue masses, obstructing the trachea and the left main bronchus, were seen. (b) Bronchoscopy showed that the left main bronchus was completely obstructed by a mass. (c) After a treatment with argon plasma coagulation (APC) and carbon dioxide cryotherapy under bronchoscopy, the left main bronchus was recanalized. (d) The biopsy pathology showed B cell non-Hodgkin's lymphoma with immunohistochemistry CD20 (+), CD56 (partially +), (magnification 40×20). (e) The repeated three-dimension reconstruction chest CT showed the left main bronchus was recanalized and the left lung returned to normal.

Discussion

Four cases of extranodal malignant lymphomas, arising from MALT in stomach, salivary gland, lung and thyroid, were firstly reported in 1984. Since then, the information about diagnosis and treatment on MALT lymphoma was gradually accumulated [1-6]. These diseases shared some clinical, histopathological and immunohistochemical features [8]. Histologically, they were characterized by noninvasive lymphoplasmacytic infiltrate, within which foci of follicle center cells can be seen invading epithelial structures, forming lymphoplasmacytic infiltrate lesions. Immunohistochemistry revealed monotypic cytoplasmic immunoglobulin in the plasma cells serving to highlight the cytoplasmic-immunoglobulin-negative lymphoepithelial lesions [8]. More importantly, they had a tendency to remain in a localized tissue for a long period and were regarded as indolent diseases with rather good survival outcomes. As for primary bronchial MALT lymphoma, the estimated 5- and 10-yr overall survival rates were 90% and 72%, respectively [7]. They had favorable responses to different kinds of therapeutic modalities, such as surgery, chemotherapy, radiotherapy, antibiotics and anti-CD20 antibody [9,10,11]. Therefore, for primary bronchial MALT lymphoma, appropriate managements to eradication of local lesions were not only possible, but also indispensable.

Considering the rarity of primary bronchial MALT, the diagnosis is extremely important for further therapeutic choice. In our two patients, the diagnosis was made on the basis of sufficient biop-

sy pathology and immunohistochemical staining. Furthermore, whole body PET/CT or CT scans provided more direct evidence to rule out the possibility of lymphomas originating or metastasis from other organs. In our two patients, bronchoscopic minimal invasive operations succeeded in acquiring a biopsy and completely erasing the lesions without significant complications. APC and carbon dioxide cryotherapy were conducted to recanalize the airway, relieve the urgent symptoms of dyspnea and earn sufficient time for subsequent systemic chemotherapy. In brief, in order to eradicate the local lesions and reduce the risk of recurrence, bronchoscopic managements for local treatment and systemic chemotherapy were elaborately combined.

MALT-derived lymphomas, having a tendency to remain localized for prolonged periods, rationalize the direct treatments for local lesions [7]. Tsurtani [12] reported a case of primary tracheal MALT lymphoma successfully treated by endoscopic neodymium-yttruim-aluminium-garnet laser photo resection and local ethanol injection. In our cases, APC and carbon dioxide frozen procedures were administered. APC was originally used for hemostasis of polypectomy under gastrointestinal endoscopy [13]. Recently, bronchoscopic APC has been used to remove malignant airway tumors, control hemoptysis and treat variety of benign diseases [14-16]. In a prospective cohort study of 364 patients who underwent APC (482 procedures), the immediate success rate was 67 percent [17]. Another retrospective cohort study found that the immediate effectiveness was

achieved in 59/60 patients by bronchoscopic APC procedure [15]. Comparing to neodymium-yttruim- aluminium-garnet laser photo resection, APC had a similar effect on resecting the lesions and a superior efficiency on achieving hemostasis. Although cryotherapy had also been used endoscopically to freeze bronchial tumors [18], there was still lack of sufficient data to confirm its effectiveness in treating tumors of the airway. In our cases, APC and cryotherapy were successful in complete resection of local MALT Lymphoma from tracheal bronchus. In summary, for bronchial MALT lymphoma, minimal invasive diagnosis and treatment under bronchoscopy has more advantages than traditional thoracic surgeries. Bronchoscopic resection of urgent life-threatening intraluminal lesions combined with systemic chemotherapy provides an optimal therapeutic choice for MALT lymphoma derived from central airway

References

- 1. Arnaoutakis K, Oo TH. Bronchus-associated lymphoid tissue lymphomas. South Med J, 2009, 102(12):1229-1233.
- Ferreri AJ, Guidoboni M, Ponzoni M, De Conciliis C, Dell OS, Fleischhauer K, Caggiari L, Lettini AA, Dal Cin E, Ieri R, Freschi M, Villa E, Boiocchi M, Dolcetti R. Evidence for an association between Chlamydia psittaci and ocular adnexal lymphomas. J Natl Cancer Inst, 2004, 96(8): 586-594.
- 3. Cadranel J, Wislez M, Antoine M. Primary pulmonary lymphoma. Eur Respir J, 2002, 20(3): 750-762.
- 4. Gaur S, Trayner E, Aish L, Weinstein R. Bronchusassociated lymphoid tissue lymphoma arising in a patient with bronchiectasis and chronic Mycobacterium avium infection. Am J Hematol, 2004, 77(1): 22-25.
- 5. Schollkopf C, Melbye M, Munksgaard L, Smedby KE, Rostgaard K, Glimelius B, Chang ET, Roos G, Hansen M, Adami HO, Hjalgrim H. Borrelia infection and risk of non-Hodgkin lymphoma. Blood, 2008, 111(12): 5524-5529.
- Chanudet E, Adam P, Nicholson AG, Wotherspoon AC, Ranaldi R, Goteri G, Pileri SA, Ye H, Müller-Hermelink HK, Du MQ. Chlamydiae and Mycoplasma infections in pulmonary MALT lymphoma. Br J Cancer, 2007, 97(7): 949-951.
- Borie R, Wislez M, Thabut G, Antoine M, Rabbat A, Couderc LJ, Monnet I, Nunes H, Blanc FX, Mal H, Bergeron A, Dusser D, Israel BD, Crestani B, Cadranel J. Clinical characteristics and prognostic factors of pulmonary MALT lymphoma. Eur Respir J, 2009, 34(6):1408-1416.

- 8. Isaacson P, Wright DH. Extranodal malignant lymphoma arising from mucosa-associated lymphoid tissure. Cancer, 1984, 53(11): 2515-2524.
- 9. Yuji Ishimatsu, Hiroshi Mukae, Kiyoshi Masumoto, Harada T, Hara A, Hara S, Amenomori M, Fujita H, Sakamoto N, Hayashi T, Kohno S. Two cases with pulmonary mucosa-associated lymphoid tissue lymphoma successfully treated with clarithromycin. Chest, 2010, 138(3): 730-733.
- Bilici A, Seker M, Ustaalioglu BB, Canpolat N, Salepci T, Gumus M. Pulmonary BALT lymphoma successfully treated with eight cycles weekly rituximab: report of first case and F-18 FDG PET/CT images. J Korean Med Sci. 2011, 26(4): 574-576.
- 11. Troch M, Strenbel B, Petkov V, Turetschek K, Chott A, Raderer M. Does MALT lymphoma of the lung require immediate treatment? An analysis of 11 untreated cases with long-term follow-up. Anticancer Res, 2007, 27(5B): 3633-3637.
- 12. Junji Tsurutani, Akitoshi Kinoshita, Hideyuki Kaida, Fujii H, Narasaki F, Fukuda M, Oka M, Kohno S.. Bronchoscopic therapy for mucosa-associated lymphoid tissue lymphoma of the trachea. Internal medicine, 1999 38(3): 276-278.
- 13. Grund KE, Storek D, Farin G. Endoscopic argon plasma coagulation (APC) first clinical experiences in flexible endoscopy. Endosc Surg Allied Technol, 1994, 2(1): 42-46.
- Crosta C, Spaggiari L, De Stefano A, Fiori G, Ravizza D, Pastorino U. Endoscopic argon plasma coagulation for palliative treatment of malignant airway obstructions: early results in 47 cases. Lung Cancer, 2001, 33(1): 75-80.
- 15. Morice RC, Ece T, Ece F, Keus L. Endobronchial Argon Plasma Coagulation for Treatment of Hemoptysis and Neoplastic Airway Obstruction. Chest, 2001, 119(3):781-787.
- 16. Vonk-Noordegraaf A, Postmus PE, Sutedja TG. Bronchoscopic treatment of patients with intraluminal microinvasive radiographically occult lung cancer not eligible for surgical resection: a follow-up study. Lung Cancer, 2003, 39(1): 49-53.
- 17. Reichle G, Freitag L, Kullmann HJ, Prenzel R, Macha HN, Farin G. Argon plasma coagulation in bronchology: a new method-alternative or complementary. Pneumologie, 2000, 54(11): 508-516.
- 18. Mathur PN, Wolf KM, Busk MF, Briete WM, Datzman M. Fiberoptic bronchoscopic cryotherapy in the management of tracheobronchial obstruction. Chest, 1996, 110(3):718-723.

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Motives to quit smoking: Insight from the melen study

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Abstract

Background: Ethnic, cultural and social factors influence the motives to quit smoking. Our aim was to investigate the current prevalence of smoking and motives of smoking cessation in Turkey, in a large population-based epidemiologic study.

Materials and Methods: A total of 2298 subjects living in Melen valley, which is inhabitant of 21 000 people were interviewed. The subjects reported information regarding socio- economic status, medical history and current use of medications. Tobacco use behaviors (current status of smoking, number of cigarettes smoked daily, duration of smoking, age of addiction, attempts and desire to quit) and motives of quitting were asked.

Results: Smoking rate was 17% and 18% of the cohort had quitted smoking. Mean age of the population was 50. Starting age of smoking was 18 ± 6 . The most frequent motive was the smokers' health status. The most common motive in primary prevention (in patients without any concomitant diseases) was the assistance of a physician. Religious belief and practice showed better success rate. Age and existence of chronic diseases (including smoking related diseases) were found to be the independent predictors of quitting (Odds ratio (OR): 1.03 [95% confidence interval (CI): 1.02-1.05], p: <0.001 and OR 2.1 [95% CI 1.37- 3.18] p:<0.001; respectively.

Conclusions: Smoking ban, cost, physician assistance, comorbidities, notices on packages, religious belief and care for family members are the most important motives to quit. The most common motive in primary prevention was the assistance of a physician and the most powerful motive was religious belief.

Key words: Cigarette, smoking, quit, relapse.

Introduction

Cigarette smoking is the most important preventable cause of morbidity and mortality all over the world. Exposure to secondhand smoke is also associated with increased morbidity and mortality in both genders and globally at all ages [1]. Smoke-free policies, when implemented, lead to a substantial and rapid reduction in tobacco-related diseases. National health campaigns related to smoking quit have begun in Turkey in the late 1990s. Advertising was prohibited by the Law on Prevention of Harmful Effects of Tobacco and Tobacco Products (Law No. 4207) in 2002. Smoking has been banned in public places (workplaces, schools, public transports) in 19th of July, 2009. The effect of these rules, regulations and campaigns on smoking cessation has not been evaluated extensively.

Reducing tobacco use is a crucial national public health issue in Turkey because the prevalence was reported among the highest in the world [2]. The majority of smokers try to quit repeatedly with periods of relapses and remissions [3]. Therefore, tobacco-control efforts should focus on giving smokers professional help to quit. It appears of great importance to recognize smokers' motivation to quit. A growing body of literature indicates that motives to quit can be divided into health, social and financial concerns, and health concern is the most important reason to quit, followed by social considerations [4]. However, ethnic, cultural and socio-demographic factors reflect significant differences between societies [5,6]. Therefore, further understanding which factors influence smokers' motivation to quit is necessary for the implementation of effectively tailored smoking cessation interventions.

The aim of this article was to investigate the current prevalence of smoking and motives to quit, effect of rules, regulations, campaigns and cultural factors on smoking cessation in Turkey in a large population-based epidemiologic study.

Methods

Study cohort

The rationale and methodology of the study was published before7. Melen is a geographic valley in north-east of Duzce, Turkey which is inhabitant of 21 000 people. There is a town centre (Yigilca) and 37 villages. Health service of the region was supplied by six family physicians, each following up almost 2500 adults. The study was conducted in May and June, 2010 in the Social health center located in the town center. 400 subjects from each family physician was randomly assigned and invited to participate the study. A total of 2298 subjects with a mean age of 50 (age range 18 to 92) were interviewed. The subjects reported information regarding socio- economic status, medical history and current use of medications. Tobacco use behaviors (current status of smoking, number of cigarettes smoked daily, duration of smoking, starting age of smoking, attempts and desire to quit) and motives of quitting were extensively asked. Marital status was classified into two categories: unmarried (including widowed or divorced) and married. Subjects living in the town center were accepted as urban living [7].

The study protocol was approved by the Ethics Committee of Duzce University (approval number: 2010/7) and every subject signed a consent form.

Interview

Smoking was defined as the constant use of at least 5 cigarettes during the 30 days preceding the interview. There were no nondaily or occasional smokers. Former smokers were defined as smoking previously at least 5 cigarette/day but who have no longer been smoking for at least six months. Attempt to quit was defined as abstaining from cigarettes for at least one month with a definite intention to quit forever. Smoking cessation was defined as abstinence for six months for persons who had smoked daily. Motivation was defined as the primary reason a person quits. The participants were asked how health campaigns, advertising and smoking ban affected their quitting behavior. Subjects who had succeeded to quit and the ones with a previous quit attempt were asked the question: "What was the most important / dominating factor for your attempt to quit? Was it the smoking ban, notices on the packages, your physicians' advice and encouragement, economic reasons / cost or comorbidities, a chronic disease that urged you to stop or another reason?" Subjects were directed to choose just one single answer. Subjects who could not express a single motive or declare numerous themes were accepted as "all". The motives that were expressed under "other" were further evaluated

Statistical Analyses

Statistical Package for Social Sciences software (SPSS 12, Chicago, IL, USA) was used for analysis. Values are given as mean \pm standard deviation. Unpaired Student's t test was used for group comparisons. Categorical data were compared with the chi-square test or Fisher's test for samples of less than five. Stepwise multivariate logistic regression analysis was used to determine which motives predicted quitting best. The covariates considered were age, gender, starting age of smoking, marital status, place of living, employment status and existence of chronic diseases. A p value of < 0.05 was considered significant.

Results

A total of 2298 subjects (1471 female, 827 male with a mean age of 50) were interviewed. Sixty five percent of the study population (1495 subjects) had never smoked. Three hundred eighty nine subjects were current smokers where as 414 subjects had quitted smoking. Smoking rate of the population was 17%. Quit rate was 18%. Starting age of smoking was 18±6. The success of quit had increased recently. The characteristics of the current and former smokers were shown in Table 1. Subjects who succeeded to quit were significantly older and richer. There was also a significant difference between the two group regarding urban living, gender, marital status and frequency of chronic diseases.

Among the smokers 620 subjects had at least once attempted to quit; 414 of these had quitted smoking and 206 had relapsed. Among the active smokers only 63 (16%) did not want to quit. One hundred eighty three (47%) current smokers had the desire to quit but had not tried.

The motives of quitting were shown in Table 2. The most frequent motive was the smokers' health status (having a chronic disease that urged the patient to use drugs daily and continuously). The most common motive in primary prevention (in patients without any concomitant diseases) was the assistance of a physician. Mean success rate was 67%. It is especially higher in male pati-

Table 1. Comparison of cohort characteristics	according to the smo	king status	
	Active smokers (N: 389)	Former smokers (N:414)	P value
Age (yrs)	44±14	53±15	< 0.001
Gender (Female)	153 (39%)	82 (20%)	< 0.001
Family income (Turkish lira/month)	878±726	907±691	< 0.001
Marital status (married)	336 (87%)	379 (92%)	0.024
Place of living (urban)	103 (27%)	75 (18%)	0.004
Amount of smoking (pack/year)	23±19	27±27	0.131
Chronic disease	104 (27%)	198 (48%)	< 0.001

Table 2. Motives for qui	itting smoking	and relative su	<i>access rate</i>						
	Total Attempt (N:620)	Total Success (N:414)	Total Relative success rate (%)	Men Attempt (N:620)	Men Success (N:414)	Men Relative success rate (%)	Women Attempt (N:161)	Women Success (N:82)	Women Relative success rate (%)
Smoking ban	35 (6%)	14 (3%)	40	28 (6%)	12 (4%)	43	7 (4%)	2 (2%)	29
Warning notice	37 (6%)	11(3%)	30	26 (6%)	9(3%)	35	11 (7%)	2 (2%)	18
Doctor's assistance	44 (7%)	28 (7%)	64	28 (6%)	20 (6%)	71	16 (10%)	8 (10%)	50
Chronic disease	230 (37%)	151(37%)	66	177 (39%)	126 (38%)	71	53 (33%)	25 (31%)	47
Religion	15 (2%)	14 (3%)	93	14 (3%)	13 (4%)	93	1 (1%)	1 (3%)	100
Cost	21 (3%)	10 (2%)	48	9 (2%)	6 (2%)	66	12 (8%)	4 (5%)	33
Pregnancy / child	7 (1%)	4 (1%)	57	1 (0,2%)	1 (0,3%)	100	6 (4%)	3 (4%)	50
Spouse	2 (0.3%)	0	0	1 (0,2%)	1 (0,3%)	100	1 (1%)	1	100
All	229 (37%)	180 (44%)	77	175 (38%)	144 (43%)	82	54 (34%)	36 (44%)	66

ents (71% success rate). Religious beliefs showed better success rate and the most powerful motive was religion (93% success rate). Pregnancy, concern about children and effect of spouse were also powerful motives in men, but the frequency was very low (<1% overall). On the other hand, a female subject declared that she had succeeded to stop smoking with the help of her husband (Table 2).

Logistic regression analysis was done to identify independent predictors of smoking cessation (Table 3). The covariates considered were age, gender, starting age of smoking, marital status, place of living, employment status and existence of chronic diseases. Age and existence of chronic diseases were found to be the independent predictors of quitting (Odds ratio (OR): 1.03 [95% confidence interval (CI): 1.02-1.05], p: <0.001 and OR 2.1 [95% CI 1.37- 3.18] p:<0.001; respectively.

Discussion

Melen Study showed a decrease in smoking rate in Turkey. According to a nationwide study conducted in 1988, 62.8% of men and 24.3% of women aged 15 years and more were smokers [2]. Another nationwide study showed that the rate of male smokers decreased from 52% in 1990 to 39% in 2000, and in women from 26% to 24%, respectively, in adults over 30 years old8. In the WHO Statistical Information System, the prevalence of current tobacco use among adults living in Turkey was shown as 45.1% in males and 14.6% in females, 31.2% in total (WHO Statistical Information System 2005. The present survey showed a much lower rate of active smoking which was 17%. The difference is possibly due to higher rates of quit and lower rates of start.

Effect of law: In 19th of July, 2009 the biggest campaign against smoking has been started in Turkey with the motto "Smoke-free air zone" and smoking has been banned in all public places. This motivated some of the smokers to quit. The classical comment was "I could not smoke even in cafes". "This is unbearable." The effect of smoking ban on the motivation to quit smoking has also been reported before [6]. Smokers want to overcome their addiction as they could no longer bear the social and legal constraints of smoking.

Effects of notices and warnings: Our survey showed that especially notices with photographs were a powerful motive for smokers. The photographs were obligatory since 1st of January, 2010. The characteristic comment was "I was not aware of how much harmful smoking is. I was shocked when I saw the pictures on the packages". However in our study the success rate of photographs as a motive was the worst among the motives. Notices and warnings on cigarette packages might be important predictors of quit attempts for at least two reasons. First, smokers may go through a rational decision process, focusing on their perceived risks of smoking-related illnesses and deciding to quit in order avoid such consequences. Second, smokers who consider smoking-related health issues may begin to worry about the consequences of smoking and worrying may prompt the decision to quit [8,9]. Health information on the packages should take up at least 50% of the package and should display a graphic image with the written warning[10]. Physician support and assistance: Physicians play an important role in convincing the patient to quit. Furthermore they can guide the patients to use pharmacological therapy against nicotine addiction. The guideline on the clinical treatment of tobacco use developed by the US

	Odds Ratio	95 % Confidence interval	P value
Male	1.28	0.74-2.22	0.369
Age	1.03	1.02-1.05	< 0.001
Amount of smoking (pack/year)	0.99	0.96-1.02	0.525
Marital status (married)	1.28	0.68-2.38	0.45
Place of living (urban)	0.74	0.47-01.15	0.183
Employment (unemployed)	0.64	0.38-1.09	0.101
Chronic disease	2.1	1.37-3.18	< 0.001

Table 3. Logistic regression analysis of predictors of smoking cessation

Public Health Service (USPHS) suggests implementation of the "5As" approach: Ask (about tobacco use); Advise (users to quit); Assess (interest in quitting); Assist (the quit attempt); and Arrange (follow up) [11]. The characteristic comment in our survey was "I could not succeed it without my doctor's motivation." The most important issue about this motive in Turkey is the high smoking rate among physicians [12]. Physicians can serve as role models for the patients and also promote cessation services in healthcare settings. Therefore interventions to reduce smoking among healthcare providers should be an important step in any cessation strategy.

Comorbidities and health concern: Data from a recent review of over thirty retrospective studies on motivation to quit smoking, indicate that health is the most important reason for quitting smoking [4]. A recently published study showed that many smokers are not successful in quitting before the occurrence of a real health problem [5]. Health status is a major reason for quitting attempts, but thereafter smokers may benefit little. Abdullah et al showed that receiving assistance for mobility predicts quitting where as having health problems predicts intention to quit smoking in the elderly [13]. Age and existence of chronic diseases were found to be the independent predictors of quitting in the current study. However, relapse rate was relatively high indicating that elderly smokers need a multifactorial treatment program to stop smoking. Health care professionals can play a pivotal role in the promotion of a smoking cessation treatment program to elderly people.

Religion: One of the most striking finding of the current study was about religious faith and practice. There was no direct question regarding the impact of religion in the standard questionnaire and the effect was mentioned in the "other motives" section by 15 subjects. The comments were as follows; "I went to Hajj and a hajji should not smoke, therefore I quit smoking" or "I learned that smoking is strictly forbidden in religion". Another important finding of the current study was the relative success rate of the religious beliefs. The religious motive has been reported in several ethnic groups. Williams et al showed in a predominantly African American sample of older adults that subjects who reported less frequent participation in organized religious activities were also more likely to smoke (Odds Ratio=2.04, 95% CI= 1.17 -50.38)]14]. Furthermore, the positive effect of faith is not limited to a single religion. Religiousness in different faiths is associated with less use of tobacco [15-17]. However; tobacco use by religious professionals is common in Turkey which potentially limits the efficacy of this motive [18].

Cost and economic reasons: Cost was reported as one of the most important reason for quitting [5,6,19]. Smokers with lower socioeconomic status were more likely to report cost and current health problems as triggers compared with smokers with high income [20,21]. Future tobacco prevention should focus on high cost of smoking at smokers with low socioeconomic status.

Pregnancy or concern about children: Although pregnancy is a good way of motivation to quit its relative frequency is low in the general population][4]. Concern about children's health is a more general attitude. They stated that, they felt "ashamed" and "guilty" when they smoked beside their children. The thought that one gives harm to his/ her child while smoking, was very disturbing for them. Effect of spouse: Very few subjects in the current study have stated that they succeeded to quit due to their spouses support. The most common complaint from their partners was the bad breath and mouth odor.

Limitations of the study: The prevalence of current smokers was lower than the rate given in WHO statistical information system indicating a potential bias in study population (more females than males). The prevalence in the current study may not reflect the whole population in Turkey.

Conclusion

Prevalence of smoking is decreasing in Turkey. Smoking ban, cost, physician assistance, co morbidities, notices on packages, religion and care for family members are the most important motives to quit. Smoking is a world-wide health problem and every society has its own motives for quitting. All opportunities to promote tobacco control in the society should be used. Evidence based data for every nation is vital for interventions and campaigns against smoking addiction.

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References

- 1. Katsiki N, Hatzitolios AI, Mikhailidis DP. Passive smoking: the democratic right of nonsmokers to survive. Angiology. 2011; 62: 520-2
- Bilir N, Dogan BG, Yildiz AN. Smoking Behaviour and Attitudes (Ankara-Turkey), Hacettepe Public Health Foundation Publ. No. 8, ISBN 975-94618-1-1, pp19-21, Ankara 1997
- 3. Fiore MC, Novotny T, Pierce JP, Giovino GA, Hatziandreu EJ, Newcomb PA, et al. Methods used to quit smoking in the United States. Do cessation programs help? JAMA. 1990; 263: 2760-5.
- 4. McCaul KD, Hockmeyer JR, Johnson RJ, Zetocha K, Quinlan K, Glasgow RE. Motivation to quit using cigarettes: A review. Addict Behav. 2006; 3: 42-56.
- 5. Sieminska A, Buczkowski K, Jassem E, Lewandowska K, Ucinska R, Chelminska M. Patterns of motivations and ways of quitting smoking among Polish smokers: a questionnaire study. BMC Public Health. 2008; 8:274.
- 6. Baha M, Le Faou AL. Smokers' reasons for quitting in an anti-smoking social context. Public Health. 2010; 124: 225-31.
- MELEN Investigators. MELEN Study: Rationale, Methodology and Basic Results. Eur J Gen Med 2011; 8: 308-13
- 8. Onat A, Baflar Ö, Erer B. Prevalence, Relationship to HDL and Impact on Coronary Events of Smoking in Turkish Adults. Arch Turk J Card 2001; 29: 493-8.
- 9. Magnan RE, Köblitz AR, Zielke DJ, McCaul KD. The effects of warning smokers on perceived risk, worry, and motivation to quit. Ann Behav Med. 2009; 37: 46-57.
- Peters E, Romer D, Slovic P, Jamieson KH, Wharfield L, Mertz CK, et al. The impact of acceptability of Canadian-style cigarette warning labels among U.S. smokers and nonsmokers. Nicotine Tob Res 2007; 9: 473–81.
- Fiore MC, Bailey WC, Cohen SJ. Treating tobacco use and dependence. Clinical practice guideline. Rockville, MD: US Department of Health and Human Services, 2010. http://www.surgeongeneral. gov/tobacco/treating_tobacco_use.pdf

- 12. Gokirmak M, Ozturk O, Bircan A, Akkaya A. The attitude toward tobacco dependence and barriers to discussing smoking cessation: a survey among Turkish general practitioners. Int J Public Health. 2010; 55: 177-83.
- 13. Abdullah AS, Ho LM, Kwan YH, Cheung WL, McGhee SM, Chan WH. Promoting smoking cessation among the elderly: what are the predictors of intention to quit and successful quitting? J Aging Health. 2006; 18: 552-64.
- Williams CD, Lewis-Jack O, Johnson K, Adams-Campbell L. Environmental influences, employment status, and religious activity predict current cigarette smoking in the elderly. Addict Behav. 2001; 26: 297-301.
- 15. Whooley MA, Boyd AL, Gardin JM, Williams DR. Religious involvement and cigarette smoking in young adults: the CARDIA study (Coronary Artery Risk Development in Young Adults) study. Arch Int Med 2002; 162: 1604-10.
- 16. Sperber AD, Peleg A, Friger M, Shvartzman P. Factors associated with daily smoking among Israeli adolescents: a prospective cohort study with a 3-year follow-up. Prev Med. 2001; 33: 73-81.
- Saeed AA, Khoja TA, Khan SB. Smoking behavior and attitudes among adult Saudi nationals in Riyadh City, Saudi Arabia. Tobacco Control. 1996; 5: 215-9.
- 18. Acik Y, Sezer RE, Karaman F, Sezer H, Oguzoncul F, Dinc E, et al. Smoking among religious professionals in Turkey. Tobacco Control. 1998; 7: 326-7.
- 19. Eiser JR, van der Plight J, Raw M, Sutton SR. Trying to stop smoking: Effects of perceived addiction, attributions for failure, and expectancy of success. J Behav Med. 1995; 8: 321–42.
- 20. Pisinger C, Aadahl M, Toft U, Jørgensen T. Motives to quit smoking and reasons to relapse differ by socioeconomic status. Prev Med. 2011; 52: 48-52.
- 21. Vangeli E, West R. Sociodemographic differences in triggers to quit smoking: findings from a national survey. Tob Control. 2008; 17: 410-5.

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Nursing care and records in enteral nutrition by nasogastric tube: Turkey sample

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Abstract

Objectives: The aim of this study was to determine the concordance of nursing care and records in enteral nutrition by nasogastric or nasoenteric tubes with expert opinions and recommendations.

Methods: Following admission to the emergency department of a university hospital between June 2010 and June 2011, the records of 102 adult patients fed by nasogastric or nasoenteric tube inpatient in intensive care unit (n=48) and surgery units (n=56) were investigated retrospectively.

Results: The average age of these patients was 56.6±19.1. Fifty-six patients were man (54.9%) and 46 women (45.1%). The period of time between admission and the beginning of enteral nutrition was 4.5 ± 3.8 days on average. The average duration of nasogastric or nasoenteric tube feeding was 9.4±7.4 days. Only the tube placement of all patients in the intensive care unit (n=48) were confirmed by radiography and the gastric residual volumes were assessed (p<0.05). During enteral nutrition, all the patients received routinely mouth care; the bedside formula container were changed every 24 hours; 20.5% of patients were not elevate the head of the bed 30-450; 22.5% received no interventions to prevent of feeding tube occlusion, and 100% had interruptions on nutrition for various reasons.

Conclusion: Most nursing interventions in enteral nutrition were found to be non-concordant with evidence-based guidelines and recommendations. It was concluded that continuous education of nurses and the use of clinical protocols as a part of the care were necessary.

Keywords: Enteral nutrition, nursing care, evidence-based practice.

Introduction

Today, enteral nutrition is an important practice, which is preferred for feeding critical patients and is used to prevent the catabolic process caused by severe diseases.¹ Enteral nutrition has a lower complication risk and higher physiological response compared to parenteral nutrition.²⁻⁴ Despite its benefits, it has probable side effects and risks.^{3,5}

During enteral nutrition through the nasogastric or the nasoenteric tube, gastrointestinal, mechanical and metabolic complications may develop.^{6,7} Most of the complications related to enteral nutrition develop when proper nursing care is not administered. Therefore, the primarily responsibility of the nurses is to prevent complications, to recognize changes in patients when complications develop, to comment and take necessary precautions, and to reporting to physician.8 Nursing care, having a key role in the success of enteral nutrition, should make nutrition easy, improve patient comfort and reduce complications.9,10 In accordance with evidence-based guidelines or recommendations, a systematic and elaborate approach is important to increase the benefits from treatment acquired and to reduce the probable risks.^{3,9} It is known that the level of nurses' information on this issue is inadequate,^{3,11,12} their responsibilities on nutritional interventions are not clear,10 and nursing care is carried out by conventional methods rather than evidence-based practice.^{5,13} It is of vital importance to carry out enteral nutrition practice according to evidence-based practice to improve nursing care quality. In Turkey, there is a limited number of studies on this subject. The aim of this study was to investigate nursing interventions on enteral nutrition through nasogastric or nasoenteric tube and related nursing records, and the assessment of their concordance with expert opinion and recommendations.

Methods

This descriptive, cross-sectional designed study was performed in a university hospital. Among 1290 adult patients presenting to the emergency

department between June 2010 and June 2011 and hospitalized in the intensive care unit and surgical units, 102 (7.8%) patients that had been fed through nasogastric or nasoenteric tube were included in the study. The medical and nursing care records of these patients were retrospectively investigated. Institutional consent was obtained prior to the study. The nurses in clinics in which the study was carried out were responsible for all the enteral nutrition practices, except for nutrient selection and feeding tube insertion. While there is no written guideline in units regarding enteral nutrition, there is a "Multidisciplinary Nutrition Committee" working in the hospital. In this committee, a physician, a dietitian, a pharmacist, and a nurse work, who provide counseling services.

Parameters evaluated in the study:

Demographic data (age, gender, presence of surgical intervention, period of stay in the units) data related to enteral nutrition (indication for enteral nutrition, days from admission to the beginning of enteral nutrition, enteral nutrition duration and type), and data related to the feeding tube (methods of test of its placement, position, number of tubes used per patient) were all obtained from medical records. Data related with nursing interventions (confirmation of tube placement, patient's body position during the enteral nutrition, frequency of bedside formula container change, frequency of nutrient formula preparation, oral and nasal skin care, prevention of tube occlusion, assessment of gastric residual volume, assessment the intolerance, interruption of tube feeding and causes, medications applied through the feeding tube) were obtained from the nursing care records. There were no exclusion criteria in this study.

Statistical analyses

The data evaluation was performed using the "SPSS for Windows 11" package program. The numerical variables were summarized as mean \pm SD and the categorical variables as numbers and percentages. The x2 test, independent samples t-test and the Pearson correlation were used to analyse relationship between various factors related to enteral nutrition. Statistical analysis was given to a value of p <0.05.

Results

The average age of the patients was 56.9 ± 19.5 (range: 19-91 ages). Fifty-six patients were man (54.9%) and 46 women (45.1%). The characteristics of 102 adult patients fed enterally have been presented in Table 1.

Variable	es	ICUn (%)	Surgicn (%)	p value
Age (years)	x±SD	56.5±18.4	56.7±19.8	p>0.05
Condor	Female	18 (17.6)	28 (27.5)	n>0.05
Gender	Male	30 (29.4)	26 (25.5)	p=0.03
Surgical intervention	Yes	39 (38.2)	38 (37.3)	n>0.05
Surgical intervention	No	9 (8.8)	16 (15.7)	p=0.03
Indication for tube feeding	Support treatment	33 (32.4)	41 (40.2)	n>0.05
Indication for tube feeding	Malnutrition	15 (14.7)	13 (12.7)	p=0.03
Discoment of tube	Gastric	30 (29.4)	54 (52.9)	m<0.001
Placement of tube	Jejunal	18 (17.7)	-	p<0.001
Type of nutration	Intermittent	30 (29.4)	54 (52.9)	n<0.001
Type of nutration	Continious	18 (17.7)	-	p<0.001
Number of tubes used per	1	42 (41.2)	52 (51)	n>0.05
patient	2	6 (5.9)	2 (1.9)	p=0.03
Initiation time of enteral	x±SD	53 ± 45	38 ± 2.9	n>0.05
nutrition (days)		0.0 - 1.0	5.0 - 2.7	P 0.00
Duration of enteral	x+SD	99 + 77	89 + 72	n>0.05
nutrition (days)	A=0D	<i>y.y</i> ± <i>1.1</i>	0.7 ± 7.2	P ⁻ 0.05
Lenght of hospital stay	x+SD	17.1 + 9.7	139 + 99	n>0.05
(days)	A±0D	17.1 - 7.7	15.7 ± 7.7	p- 0.05

Table 1. The characteristics of patients who fed enterally (N=102)

*ICU=Intensive care unit

The period of time between admission and the beginning of enteral nutrition was 4.5 ± 3.8 (range: 1-23 days) days on average. The average duration of nasogastric tube feeding was 9.4 ± 7.4 days, ranging from 3 to 34 days. The patients stayed in the units an average of 15.4 ± 9.9 days (range 4-50 days). When compared with intensive care unit, patients in surgical units were started on enteral nutrition earlier. Although the nutrition duration and the length of hospital stay were shorter, there was no significant difference (p>0.05) (Table 1). The initiation time of enteral nutrition and duration of enteral nutrition were found to be positively correlated with the length of hospital stay (r=0.638, r=0.842, respectively) (p<0.001).

Immediately after insertion of the tube by the physicians, confirmation of tube placement was carried out by radiography in all patients in intensive care unit (n=48; 47.1%), and by gastric content aspiration and auscultation in all patients in the surgery units (n=54; 52.9%). During enteral nutrition, there was no information related with secondary method to confirm placement of feeding tubes by nurses in any of the patient records. The applied and the recorded nursing interventions have been presented in Table 2.

In this research was the presentation of medicines administered by tube, 40 patients (39.2) in the intensive care unit and 36 patients (35.3%) in the surgical units receiving one or more drugs in pills

	Recorded i	nterventions
Due sties /interview /server ants	ICU	Surgical units
Practice/intervention/comments	(n=48)	(n=54)
	n (%)	n (%)
Confirmation of tube placement		
Radiography	-	-
Mark the feeding tube with indelible ink at the exit side		
from the lip or naris	-	-
Check this mark before feeding or administering		
medication	-	-
Body position		
Elevate the head of the bed 30-45°	37 (36.3)	44 (43.1)
Assess of gastric resudual volume	48 (47.1)	-
Assess of gastrointestinal intolerance	48 (47.1)	54 (52.9)
Routinely oral and nasal care	48 (47.1)	54 (52.9)
Avoidance of bacterial contamination		
Wipe top of formula cans with alcohol routinely change	-	-
bedside formula container every 24 hours	48 (47.1)	54 (52.9)
Replace formula every 4 hours in open feeding systems	48 (47.1)	54 (52.9)
Storage in a refrigerator of opened formula	-	-
Prevention of tube occlusions		
Routinely flush tube with water	37 (36.3)	42 (41.2)
Flush tube with 30 ml every 4 hours	4.7±3.7	8.1±4.1
Treatment of tube occlusions		
If flushing with warm water is ineffective, use pancreatic		
enzyme solution	-	-
Interruptions of feeding	48 (47 1)	54 (52.9)
Stop enteral nutrition immediately before minor	40 (47.1)	54 (52.7)
procedures and restart within 1 hour after procedure	_	_
Avoid stopping enteral nutrition for more than 4 hours	_	
before major procedures	-	-

Table 2. Nursing interventions related to enteral nutrition (N=102)

or capsules (that crushing or dissolving before administration). Drugs administered simultaneously with enteral nutrition were antihypertensives in 50.9% (n=52), antiepileptics in 19.6% (n=20), antipsychotics in 17.6% (n=18), analgesics in 15.7% (n=16), and anticoagulants in 11.6% (n=12).

Discussion

Although there is no data related to early enteral nutrition having improved the results, The European Society for Clinical Nutrition and Metabolism (ESPEN) recommends beginning of nutrition in all critical patients who are hemodynamically stable and normal gastrointestinal system functions in 24-48 hours.^{1,14} The results of this study showed that the period of time between admission and the beginning of enteral nutrition in 102 patients was later than the recommendations of ESPEN, particularly in intensive care unit patients. This condition may be related to unstable hemodynamic conditions and abnormal gastrointestinal system functions. Thus, 77 patients (75.4%) had undergone surgical interventions (Table 1). In the study of Gupta et al.¹⁵, it was determined that enteral feeding was not begun early in intensive care units and nutrition was delayed. Whereas critical patients need early and specific nutrient support and have high metabolic requirements.¹⁶ Early start of enteral nutrition support provides gastrointestinal system function and integrity, thereby decreasing the translocation in microorganisms. These positive benefits result in decreased number of complications, decreased length of hospital stay and lowered risk of death.¹⁵

In the present study, there was a positively significant relation between the beginning time of enteral feeding and enteral feeding duration, and the length of hospital stay (p<0.001). Furthermore, in previous studies, starting nutritional support more than 3 days after admission to the intensive care unit was associated with an increased length of stay;¹⁷ the prolongation of the nutrition duration was significantly related to the length of hospital stay.⁶

The most important complication related with enteral nutrition was oropharyngeal or gastric content aspiration.⁵ Aspiration pneumonia is among the most common causes of death and its incidence varies between 5% and 58%.⁷ To prevent aspiration with high mortality, the tube insertion site should be confirmed,^{3,6,7,17,19} the head of the bed should be elevated during enteral nutrition,^{3,5-7,13,17,19-21} gastric residual volume should be checked,^{3-5,7,17,20,22} and routinely oral care^{5,6} is recommended.

Studies have shown that malposition of feeding tubes range between 1.9% to 89.5%,23 and that the auscultation method and gastric content aspiration provided a false safety in confirming of tube placement;^{3,17,23} malposition of the tube is a factor related to an increased aspiration risk.^{5,17} The American Gastroenterological Association (AGA) has recommended that radiography is direct evidence for confirmation of tube placement.^{3,6,7,17} The American Association of Critical Care-Nurses (AACN) recommends using a segondary methods to confirm placement of feeding tubes. The method consists of marking the feeding tube with indelible ink at the exit site from the lip or naris at the time of radiography.¹⁷ This mark must be confirmed by a nurse before feeding or administering medications through the feeding tube.^{9,17,19} In spite of these recommendations, it is a notable finding that the tube placement was confirmed by radiography in all intensive care unit patients, and by auscultation in all surgery patients. Furthermore, there were no records indicating that the tubes were marked by a indelible ink and the tube placement was checked by nurses before feeding or medication administrations (Table 2). This may be a result of the perception that there is no obligation to record this information or that reliable methods directed to prevention or control of aspiration risk are not put into practice.

The American Gastroenterological Association recommends elevating the head of bed to a minimum of 300 to 450 to reduce the risk of microaspiration.^{3,5-7,17,19-21} Furthermore, it is recommended that this position should be kept at least for one hour after feeding has been completed.^{7,22,24} In this study, while 79.4% (n=81) of the patients were given a 30-45 degrees semi-recumbent position during feeding, 20.6% (n=21) laid in the supine or lateral position during feeding. After nutrition was completed, there were no records whether the patients were further kept in an elevated position at least for one hour or not (Table 2). This may be a result of the inadequate knowledge levels of nurses related with the enteral nutrition and absence of a clinical protocol. Knowledge levels related with enteral nutrition showed a significant difference according to the education levels and the clinical experience of the nurses.¹⁸ In another study, in units where clinical protocols guiding the nurses were found, the mortality rate was seen to have decreased by 10% compared to that without protocols³. Kenny and Goodman¹³ reported that tube feeding education increased the knowledge levels of nurses and all the nurses placed all the patients in a proper position during nutrition. Another factor that causes an increased aspiration risk related with enteral nutrition is high gastric residual volume. Many factors such as opioids, surgical intervention, trauma, shock and respiratory failure decrease the gastric motility. High gastric residual volume delays the gastric emptying and leads to gastrointestinal intolerance, regurgitation and aspiration risk. Therefore, gastric residual volume assessment is important.^{3,5,7,17,19,20} In the present study, the nurses checked and recorded the gastric residual volume only in all the intensive care unit patients (Table 2).

This shows the knowledge and practice difference of nurses related with the enteral nutrition. In the literature, it has been reported that the nursing care of patients fed by the enteral route was performed using the conventional methods rather than the evidence-based practice; differences were observed in both the in-facility and between the institutions with regard to care procedures.¹³ In a study in a university hospital of Turkey with a Nutrition Support Unit, 282 applications performed by 26 nurses were observed and all the observations showed that nurses routinely checked the feeding tube placement and gastric residual volume.⁸ In another study on three different hospitals without clinical protocols, the knowledge levels of nurses regarding enteral nutrition, their perception of responsibilities, the records and the practices were investigated. As a result, the nurses' source of information was determined to be their colleagues in their units; none of the nurses evaluated the gastric residual volumes, and the nursing practice in each institution was different.25

Another risk factor that may be modified with regard to reducing the aspiration risk is oral hygi-

ene. In the literature, aggressive oral care has been reported to reduce the risk of aspiration pneumonia by 60%.²⁴ In this study, all patients received routinely oral care in accordance with the recommendations^{5,24} (Table 2).

One of the practices to which attention should be paid in patient care with enteral tube feeding is assessment of gastrointestinal intolerance (complications such as nausea, vomiting, gastric distention, diarrhea, constipation).^{5,7,16,19,26} In the present study, gastrointestinal intolerance was followed and recorded in all patients according to the recommendations (Table 2). While some of gastrointestinal complications commonly seen in patients in whom enteral feeding was administered were not directly correlated with the nursing care, the role of nurses is to aid in preventing and recovering of the complications. Therefore, it is important that nurses should evaluate the patients regularly for signs of intolerance and report to physician for early diagnosis and treatment.^{6,19,27}

Enteral nutrients are ideal media for bacterial growth.²¹ Bacterial contamination of the gastrointestinal tract during enteral nutrition may also led to intolerance.7,17 Aseptic techniques, such as hand hygiene,⁷ cleaning the tops of formula cans with alcohol swabs before opening,¹⁷ routinely changing the bedside container of formula every 24 hours,^{6,7,9,17,21} replacing formula every 4 hours in open systems,^{9,17} storing in the refrigerator of open formulas,⁷ are used to reduce bacterial contamination. There is no reliable evidence that closed system feeding is better than the open system.^{9,17} In this study, concordant with the recommendations, the bedside formula container were regularly changed every 24 hours by nurses, but the nutrient formula was replaced every eight hours instead of four (Table 2). There was no record related with hand hygiene, clean the tops of formula cans with alcohol swabs and the storage conditions of the open formula. This condition may be explained by lack of regular records directed to nursing care, absence of obligation to record this information or ignorance of the relation between these practices and possible complications. In a study investigating the practice and records directed towards the enteral feeding practice of nurses in Turkey, there were unrecorded applications and the most important cause for absence of the records was excess workload.8

One of the important practices of patient care in enteral nutrition is preventing tube occlusion. Occlusions inside feeding tubes are often caused by coagulation of protein-based formulas as the comes in contact with acidic environments or certain medications. An increase in tube occlusions is also associated with the performance of gastric residual checks.⁹ To maintain tube patency, the feeding tube should be washed with 20-100 ml warm water every four hours or after per medication administration;^{7,9,17,21} in case of occlusion, if washing with warm water is inadequate, pancreatic enzyme solution should be used,4,9 administration of pills or capsules form medications should be avoided,^{17,21} if medications must be administered through the feeding tube, liquid forms should be preferred.²¹ It was found that the feeding tubes of all patients in this study were washed by 20-100 ml water, but the frequency of washing was longer in the surgical units $(8.1\pm4.1; \text{ range } 2-12 \text{ hours})$ than the intensive care unit $(4.7\pm3.7; \text{ range } 2-12)$ hours), and there was a significant difference (t=-3.846; p=0.001) (Table 2). It was also found that most patients received one or more medications in pills or capsules form (n=76; 74.5%) through the feeding tube. In a survey study, 33% of 1167 intensive care unit nurses administered a mean of 8.9 medications daily through the enteral nutrition tube.28 In another study on 49 nurses working at the intensive care unit, 42.8% administered the prescribed solid medications by crushing, 32.6% by dissolving in 20 ml water, 65.3% did not make any recommendations to physicians with regard to the choice of medication type.²⁹

It is recommended that interruptions during enteral nutrition should be reduced, nutrition should be continued until medical and diagnostic procedures begin, nutrition should be restarted in one hour following minor procedures and in four hours' time following major procedures as long as there is no specific contraindication, and this interruption should not last for more than four hours.^{17,19} In current study, in spite of the recommendations, nutrition was found to be interrupted in all patients for different reasons, such as care practices, diagnostic tests, impaired general status and physician requests (Table 2).

However, there was no data available regarding the time the enteral nutrition was stopped. Elpern

et al.³⁰ found that mean length of interruptions in enteral feeding was 5.23 hours per patient per day. The top 3 reasons for the interruptions were preparation for test (35.7%), changes in body positions (15%), and high gastric residual volumes (11.5%). Apart from the care practices, although the decision for interruption is not made by nurses, continuity is a part of the care and it is among the responsibilities of nurses.

Conclusion

The results of this study show that many nursing care practices are not appropriate for evidence-based guidelines and recommendations. Safe and effective enteral nutrition requires careful and elaborate medical treatment and nursing care. We recognize that evidence-based guidelines and continuous education of nurses are steps towards changing practice. When the study results are considered, clinical guidelines directed to nurses should be used as a part of care.

Study limitations

This study was performed at a university hospital. However, the results may not reflect the whole population in Turkey. Another limitation of the study was that the data depended on the recorded data and its retrospective design.

References

- 1. Kreyman KG, Berger MM, Deutz NEP, Hiesmayr M, Jolliet P, Kazandjiev G, et al. ESPEN Guidelines on enteral nutrition: Intensive care. Clin Nutr 2006;25:210-23.
- 2. Ellett ML. Important facts about intestinal feding tube placement. Gastroenterol Nurs 2006;29(2):112-24.
- 3. Heyland DK, Cahill NE, Dhaliwal R, Sun X, Day AG, McClave SA. Impact of enteral feding protocols on enteral nutrition delivery: Results of a multicenter observational study. J Parenter Enteral Nutr 2010;34(6):675-84.
- 4. Demiral U, Bahçecioğlu İH. Enteral ve parenteral beslenmeye klinik yaklaşım. Güncel Gastroenteroloji 2010;14 (3):149-54.
- 5. Williams TA, Leslie GD. A review of the nursing care of enteral feding tubes in critically ill adults: Part I. Intensive Crit Care Nurs 2004;20(6):330-43.

- 6. Pancorbo-Hidalgo PL, Garcia-Fernandez FP, Ramirez-Perez C. Complications associated with enteral nutrition by nasogastric tube in an internal medicine unit. J Clin Nurs 2001;10(4):482-90.
- 7. Gavi S, Hensley J, Cerva F, Nicastri C, Fields S. Management of feding tube complications in the long-term care resident. Annals of Long-term Care 2008;16(4): 28-32.
- Uysal N, Eşer İ, Khorsid L. Hemşirelerin enteral beslenme işlemine yönelik uygulama ve kayıtlarının incelenmesi. Anadolu Hemşirelik ve Sağlık Bilimleri Dergisi 2011;14(2):1-9.
- 9. Williams TA, Leslie GD. A review of the nursing care of enteral feeding tubes in critically ill adults: Part II. Intensive Crit Care Nurs 2005;21(1):5-15.
- 10. Persenius MW, Wilde-Larsson B, Hall-Lord ML. To have and to hold nutritional control: Balancing between individual and routine care. A grounded theory study. Intensive Crit Care Nurs 2009;25(3):155-62.
- 11. Schaller C, James EL. The nutritional knowledge of Australian nurses. Nurse Educ Today 2005;25(5):405-12.
- 12. Mowe M, Bosaeus I, Rosmussen HH, Kondrup J, Unosson N, Rothenberg E, et al. Insufficient nutritional knowledge among health care workers. Clin Nutr 2008;27(2):196-202.
- 13. Kenny DJ, Goodman P. Care of the patient with enteral tube feeding: An evidence-based protocol. Nurs Res 2010;59(1 Suppl):22-31.
- 14. Weimann A, Braga M, Harsanyi L, Laviano A, Ljungqvist O, Soeters P, et al. ESPEN Guidelines on enteral nutrition: Surgery including organ transplantation. Clin Nutr 2006;25:224-44.
- 15. Gupta B, Agrawal P, Soni KD, Yadov V, Dhakal R, Khurans S, et al. Enteral nutrition practices in the intensive care unit: Understanding of nursing practices and perspectives. J Anaesthesiol Clin Pharmacol 2012;28(1):41-4.
- 16. Roynette CE, Bongers A, Fulbrok P, Albarran JW, Hofman Z. Enteral feeding practices in European ICUs: A survey from the European federation of critical care nursing associations (EfCCNa). e-SPEN, the Eur e-Journal Crit Nutr and Metabol 2008;3(2):e33-e39.
- 17. Bourgault AM, Ipe L, Weaver J, Swartz S, O'dea PJ. Development of evidence-based quide lines and critical care nurses' knowledge of enteral feeding. Crit Care Nurse 2007;27(4):17-22.
- 18. Yun SH, Kim SJ, Oh EG. Healthcare professional's knowledge, perception and performance on early enteral nutrition for critical ill patients. Korean J Crit Care Med 2012;27(1):36-44.

- 19. Winkelman C, Best K. Formula for success: Deliver enteral nutrition using best practices. Am Nurs Today 2009;4(3):18-23.
- 20. Kattelmann KK, Hise M, Russell M, Charney P, Stokes M, Compher C. Preliminary evidence for a medical nutrition therapy protocol: Enteral feeding for critically ill patients. J Am Diet Assoc 2006;106(8):1226-41.
- 21. Stroud M, Duncan H, Nightingale K. Guide lines for enteral feeding in adult hospital patients. Gut 2003;52 (Suppl VII), vii1-vii12.
- 22. Marik PE, Kaplan D. Aspiration pneumonia and dysphagia in the elderly. Chest 2003;124(1):328-36.
- 23. Ellett ML. What is know about methods of correctly placing gastric tubes in adults and children. Gastroenterol Nurs 2004;27(6): 253-9.
- 24. Loeb MB, Becker M, Eady A, Walker-Dilks C. Interventions to prevent aspiration pneumonia in older adults: A systematic review. J Am Geriatr Soc 2003;51(7):1018-22.
- 25. Persenius MV, Larsson BW, Hall-Lord ML. Enteral nutrition in intensive care: Nurses' perceptions and bedside observations. Intensive Crit Care Nurs 2006;22(2):82-94.
- 26. Akıncı SB. Enteral nütrisyon uygulama yöntemleri. Klinik Gelişim 2011;24:20-5.
- 27. Eisenberg P. An overview of diarrhea in the patient receiving enteral nutrition. Gastronterol Nurs 2002;25(3): 95-104.
- 28. Belknap DC, Seifert CF, Petermann M. Administration of medications through enteral feeding catheters. Am J Crit Care 1997;6(5):382-92.
- 29. Mata MLS, Barbosa IV, Studart RMS, Meto EM, Lima FET, Mariano FA. Evaluation of intensivistnurses' knowledge concerning medication administration through nazo gastric and enteral tubes. Rev Lat Am Enfermagem 2010;18(5):888-94.
- 30. Elpern EH, Stutz L, Peterson S, Gurka DP, Skipper A. Outcomes associated with enteral tube feedings in a medical intensive care unit. Am J Crit Care 2004;13(3): 221-7.

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Palliative care in cancer: An overview

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Abstract

Introduction: In cancer treatment, focus eventually has to shift from curing to palliating, an approach called cancer palliative care. This literature review aimed at analyzing the existing studies on cancer palliative care on a broad perspective.

Methods: A search was conducted via electronic databases, using various combinations of the keywords Palliative Care, Neoplasms, Pain Management and Quality of Life.

Results and Discussion: Palliative care is now an accredited professional discipline. In general, palliative care models can be provided in 3 different settings: domiciliary, inpatient- or outpatient/ consultative. Cancer palliative care is a multidisciplinary task, shared by informal and formal caregivers. Cancer pain relief in palliative care is part of a comprehensive pattern of care. Due to the lack of adequate studies, limited impact on patients' quality of life has been demonstrated, although patients subjected to palliative care have the potential to have 'a good death'.

Conclusion: Palliative care on a cancer patient treatment has positive effects on patients and on the other people involved. Thus, it is important to adopt palliative care since the moment of diagnosis.

Keywords: Palliative care, neoplasms, pain management.

Introduction

Among death causes, cancer is the only one which increases in every country and continent worldwide^{1,2}. That is why cancer control is now considered a priority public health issue².

The World Health Organization (WHO) expects, by the year 2030, 27 million incident cases of cancer, 17 million deaths caused by cancer and 75 million people alive each year with the disease³. This burden is currently shared between

developed and less developed nations as, of the 10 million new cancer cases each year, 4.7 million are in the more developed countries and nearly 5.5 million are in the less developed countries². In Brazil, it is estimated by the year 2012 (and by 2013 as well) 518.510 new cases of cancer³.

Modern medicine has achieved great advances regarding cancer treatment. Still, approximately 50% of all cancer patients die from their disease, meaning that, for every second patient, focus eventually has to shift from curing the disease or prolonging life to palliating⁴.

When dealing with cancer patients facing terminality, it is important to maintain health with quality of life⁵. A good cancer care should successfully prevent or relieve suffering caused by the disease and its treatment. This achievement will bring along quantifiable outcomes for cancer patients, including prolonged and improved quality of life⁶. The approach of helping patients and their families to deal with a life-threatening illness is being called palliative care¹. Discussing end-of-life care models is relevant, as long as these models affect many lives, directly (people being taken care of) or indirectly (people attached to those being take care of). Besides, facing terminality involves coping with physical deprivation and difficulties on other areas, such as emotional and social spheres⁷. To date, a great number of studies have examined several aspects regarding palliative care. The aim of this literature review was analyzing the existing studies on cancer palliative care on a broad perspective, understanding its definition, guidelines, models, caregivers involved, pain management strategies and quality of life issues.

Methods

A search of the literature was conducted via electronic databases (LILACS, IBECS, MED-

LINE, Cochrane Library, SciELO), from inception of each database to May 2012. The search strategy employed various combinations of the following keywords: Palliative Care, Neoplasms, Pain Management and Quality of Life and their equivalents in Portuguese. An additional subset of articles was identified through reference lists of the retrieved articles. Data was also gathered from reports of national and international official health organizations, as well as from other publications, considering their value as historic reference and their relevance to the subject at study.

Results and Discussion

Definition of Palliative care

Palliative care has been defined by the World Health Organization (WHO) as an approach that 'improves the quality of life (QOL) of patients and their families facing the problem associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems, physical, psychosocial and spiritual².

Over the past few decades, palliative care has evolved from a philosophy of care to an accredited professional discipline, with a growing number of clinical programs and an accumulation of expertise related to symptom control, psychosocial and spiritual care, communication, decision making, and end-of-life care⁸.

Nonetheless, despite the improvements in the palliative care study, supportive and palliative oncology literature is still in need of definitional clarity for many important terms. 'Palliative care' and 'end-of-life' are the most frequently used terms, whereas 'supportive care' was used more often in oncology journals. It is, then, advisable to provide a definition or citation when using terms regarding palliative care⁸.

Guidelines and models of Cancer Palliative Care

In the first decade of the 21st century, several models of care have been developed to provide palliative care in about fifty countries in the world⁹. Presumably for protection matters, a special place at home or at the hospital is set for the

care of advanced cancer patient⁵. Hence, palliative care can be provided intra- and extramurally to patients with terminal illness⁹.

Palliative care models, carried either at home or at a hospital, should follow certain guidelines, in order to achieve the aims of suffering relief and quality of life improvement of terminally ill patients and their families1. These guidelines are grouped in six domains: 1) Structure and Processes of Care, 2) Physical Aspects of Care, 3) Psychological and Psychiatric Aspects of Care, 4) Social Aspects of Care, 5) Spiritual, Religious and Existential Aspects of Care, 6) Cultural Aspects of Care1,10. The National Consensus Project for Quality Palliative Care added two other domains to the list: Care of the Imminently Dying Patient (last 24-48h hours) and Ethical and Legal Aspects of Care¹⁰. In general, palliative care services can be provided in 3 different settings: domiciliary, inpatient- or outpatient/consultative. In domiciliary settings, referred to as 'hospice home care' in the United States, palliative care is delivered at the patient's home. Inpatient settings include hospices or homes for the terminally ill, units in hospitals or nursing homes, homes for the elderly and homes for terminally ill children. Finally, outpatient/consultative settings can be either intramural (such as for non-palliative units in hospitals) or transmural (e.g., to support palliative home care services)⁹.

Despite the multiple services offered, it is important to highlight that there is no ideal palliative care model. Choosing one or more models is a decision that must take into account different needs, such as population's, public and private resource providers' and health care services'1.

Caregivers in Cancer Palliative Care

The onus and rewards of taking care of a terminally ill patient can be shared by different caregivers, either informal (e.g., relatives, friends and neighbors) or formal (e.g., health care professionals) ones¹.

Palliative care is necessarily a multidisciplinary task. It is unrealistic to expect a single professional to bear the skills to make the necessary assessment, institute the necessary interventions, and provide ongoing monitoring¹¹. A multidisciplinary team¹², of physicians, nurses, nutritionists, social workers and other health care professionals must be involved in a palliative care unit (hospices or homes, hospitals or at home)¹.

The palliative care team must seek qualification to offer proper service, palliative care-oriented. They should also remember that, although they do not always have all the answers, it is important to be available to seek evidence in order to find the adequate line of action, and also to share his/her feelings with the other team members¹³. Volunteers, family, and friends also take an active part in providing support when needed⁹. Like formal caregivers, informal ones should be clarified about the patient's disease and on how to take care properly. They must receive social and psychological support as wel¹¹.

Management of cancer pain in palliative care

For most patients, physical pain is only one of several symptoms that must be addressed simultaneously. That is why it is essential to understand pain relief as part of a comprehensive pattern of care, encompassing physical, psychological, social, and spiritual aspects of suffering¹¹. Relief from cancer pain can be achieved in about 90% of patients². Adequate pain relief can be achieved with systemic medications alone in most cases. In case systemic medication fails, due to inadequate analgesia or burdensome side effects, invasive techniques may complement, or even replace, systemic therapy¹⁴. The main obstacle to pain relief in cancer is the insufficient availability of opioid drugs, because of regulatory and pricing obstacles, ignorance, and false beliefs². Recent studies claim that WHO guidelines for the treatment of cancer pain approach the problem mainly toward the end of life situation in the context of metastatic, progressive disease, whereas new data show pain to be problematic throughout the cancer care cycle. Effective treatment strategies should, then, include multidisciplinary, multimodal care utilizing:

1) combinations of long-acting opioids for constant pain with short-acting opioids for breakthrough pain;

2) "adjuvant" co-analgesics, including nonsteroidal anti-inflammatories, anticonvulsants, antidepressants, and topical agents to optimize analgesia and minimize opioid doses, thereby reducing concomitant opioid-related side effects; 3) prophylactic treatment of constipation, nausea, and other common troublesome symptoms;

4) interventional options for pain control, including nerve blocks, spinal infusions, vertebral augmentation, and other procedures. Lastly,

5) psychological evaluation and support¹⁵.

Quality of life in palliative care cancer patients

According to the World Health Organization, palliative cancer care aims at improving a patient's subjective well-being. To meet the multiple and varying needs, it is generally believed that the care should be holistic, multidisciplinary, and family-as well as patient-centered⁴.

This will demand evaluating the outcome of palliative care, which usually requires setting up special mechanisms to assess quality of life. Special studies may be conducted among patients, their families and healthcare providers considering the various dimensions of quality of life: pain relief and other symptom control, functionality, psychosocial and spiritual well-being, family and medical interaction, financial issues, and so on. There are several quality-of-life instruments available in the literature; however, very few of them were validated within palliative care populations².

Due to the lack of adequate studies, limited impact on patients' health-related quality of life (HRQL), including physical and psychosocial dimensions, has been demonstrated. Few randomized trials have been reported, and these, as well as most nonrandomized studies, have been criticized for methodological reasons⁴.

Apart from this controversy, though, there is evidence that, supported by an interdisciplinary palliative care team, most patients have the potential to achieve what literature states as 'a good death'. Factors that may affect the achievement of good death and life quality include patient characteristics, care settings and quality of care¹⁶.

Conclusions

As a result of the increasing incidence of chronic, ultimately fatal illnesses worldwide, the development of an adequate palliative care plan has evolved as an important public health issue. In this context, alarming cancer statistics set cancer control as one of the highest priorities regarding palliative care. Palliative care insertion on advanced cancer patient treatment may improve his/her quality of life, relieve suffering through symptom control and psychological and spiritual assistance and, in end of life cases, contribute to allow patient to have a 'good death', respecting the body limits and, most of all, the patients' right to have dignity in his/her last moments. Moreover, positive effects of treating cancer patients in a palliative care-oriented perspective reach not only patients, but also health care professionals, family and other persons involved.

Thus, it is essential to develop the practice, already advised by international health organizations such as WHO, of adopting palliative care since the moment of diagnosis. When dealing with cancer palliative care, the sooner practices are brought to the patient's routine, the better.

References

- 1. Silva RCF, Hortale VA. Cuidados paliativos oncológicos: elementos para o debate de diretrizes nesta área. Cad Saude Publica 2006;22(10):2055-66.
- 2. World Health Organization. National cancer control programmes: policies and managerial guidelines. 2 ed. Geneva: World Health Organization; 2002.
- 3. Instituto Nacional do Câncer (Brasil). Estimativa 2012: incidência de câncer no Brasil. Rio de Janeiro (RJ): INCA; 2011.
- 4. Jordhoy MS, Fayers P, Loge JH, Ahlner-Elmqvist M, Kaasa S: Quality of life in palliative cancer care: results from a cluster randomized trial. J Clin Oncol 2001, 19:3884-3894.
- Santos MCL, Pagliuca LMF, Fernandes AFC. Cuidados paliativos ao portador de câncer: reflexões sob o olhar de Paterson e Zderad. Rev Latino-am Enfermagem 2007 mar/abr; 15(2):350-4.
- JJ Chin, CW Ho, H Arima, et al. Integration of palliative and supportive cancer care in Asia. Lancet Oncol. 2012 May; 13(5):445-6.
- 7. Singer PA, Bowman KW. Quality care at the end of life. BMJ 2003;324:1291-1292.
- Hui D, Mori M, Parsons H, et al. The lack of standard definitions in the supportive and palliative oncology literature. J Pain Symptom Manage 2012 Mar; 43(3):582–592.

- 9. Abu-Saad HH. Palliative care: an international view. Patient Education and Counseling 2000 41: 15–22.
- 10. National Consensus Project for Quality Palliative Care. Clinical practice guidelines for quality palliative care. Pittsburgh: National Consensus Project for Quality Palliative Care; 2004.
- 11. O'Neill B, Fallon M. ABC of palliative care: principles of palliative care and pain control. BMJ 1997;315801-804.
- 12. Gandin LAA, Paulini MAS. Cuidados paliativos. Serv. Soc. Rev. 2004; 6(2).
- 13. Kruse MHL, et al. Cuidados paliativos: uma experiência. Rev HCPA 2007; 27(2):49-52.
- 14. Gavrin JR, McMenamin EM. Pain management in palliative care oncology patients. Curr Pain Headache Rep. 2008 Aug; 12(4):257-61.
- 15. Burton AW. Palliative Care and Pain Medicine Come Together to Optimally Treat Cancer Pain: What We Can Learn from the British Pain Society. Pain Medicine. 2010; 11(5): 635–636. doi: 10.1111/j.1526-4637.2010.00848.x
- 16. Leung KK, Tsai JS, Cheng SY, Liu WJ, Chiu TY, Wu CH, et al. Can a good death and quality of life be achieved for patients with terminal cancer in a palliative care unit? J Palliat Med 2010;13:1433-8.

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Pomegranate seed hydroalcoholic extract improves memory deficit due to permanent cerebral hypoperfusion /ischemia in male rats

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Abstract

Background and Objective: Cerebral hypoperfusion/ischemia (CHI) is a neurological disease where impaired cognition caused by a serial pathophysiological events. This study aimed to evaluate the effect of two weeks oral administration of pomegranate seed extract (PGSE) on passive avoidance memory after permanent bilateral common carotid arteries occlusion (2CCAO) in male adult rats.

Methods: In order to make 2CCAO as an animal model of CHI, carotid arteries were ligatured and then cut bilaterally. To evaluation of passive avoidance task, step-through latency (STL) was measured by two-way shuttle box in all rats.

Results: We found that memory was significantly impaired in rats after CHI (P<0.01). PGSE treatment significantly improved memory impairment in rats with 2CCAO.

Conclusion: Our results in present study suggest that PGSE exhibits therapeutic potential for short-term memory, which is most likely related at least in part to its antioxidative and free radical scavenging actions.

Keywords: Cerebral hypoperfusion ischemia, pomegranate seed extract, memory, rat.

Introduction

Cerebral ischemia resulted from low oxygen and glucose supply evidently decreases the formation of ATP (1-2). Damage to brain tissue resulting from cerebral ischaemia is a major cause of adult disability. It can lead to cognition problems, seizures and death (3-4). Transient global cerebral ischaemia (forebrain ischaemia), occurring during cardiorespiratory arrest in patients or in experimental animals, induces selective and delayed neuronal cell death (4-7). Pyramidal neurons in the CA1 region of the hippocampus are particularly vulnerable and die after global ischemia. Hippocampal CA1 sector injury is observed (2-4, 6-7) a few days after untreated forebrain ischemia in the rat, gerbil and human (4, 8).

Brain injury following transient cerebral hypoxia-ischemia (H-I) is mediated by many mechanisms. Treatments for protection against neuronal cell death induced by H-I and reperfusion have been developed in recent years, but none has been highly successful. A fundamental process believed to be responsible for H-I damage to neurons is excitotoxicity, triggered mainly by elevated extracellular glutamate concentration (9). This ischemia-induced release of glutamate likely occurs in man as well (10), and possibly underlies selective damage to the human hippocampus. Glutamate may cause ischemic neuronal death by acting at excitatory N-methyl-DAspartate (NMDA) receptors (11) which play an important physiological role in long-term potentiation and memory (12). Thus, the high concentration of NMDA excitatory receptors on the dendrite trees of hippocampal CA1 pyramidal cells (13) likely explains the longknown selective vulnerability of the CA 1 zone of the hippocampus to ischemic brain damage.

Reactive oxygen species (ROS) are generated within brain tissue during H-I and play a role in the development of cerebral damage. They may be directly involved in glutamate release (14) and more importantly, they may participate in the excitotoxic process itself. These oxygen species are extremely reactive and attack lipids, proteins, and nucleic acids, which results eventually in tissue injury and cell death (15). Although there is strong evidence that total destruction of hippocampal CA 1 neurons is sufficient to cause a memory deficit (16). It is still presently unclear in man and in animals to what degree subtotal ischemic hippocampal damage may occur without a memory deficit ensuing. Only the CA1 hippocampal neuronal population in the brain can cause a deficit in spatial learning and memory in the rat (17). The free radicals are neutralized by an elaborate antioxidant defense system consisting of enzymes and numerous non-enzymatic antioxidants, including vitamins A, E and C, glutathione, ubiquinone, and flavonoids (18-19). Polyphenols, molecules that are defined by the presence of an aromatic ring bearing one or more hydroxyl substitutes are products of plant metabolism (20). These compounds have been found to possess antioxidant properties as well as to have effects on gene expression. Recent studies indicate that among foods that contain polyphenols, juice extracted from the pomegranate has the highest concentration of measurable polyphenols (21-22). The pharmacologic actions of pomegranate juice include antiatherosclerotic, antibacterial, and antiproliferative properties (23-24). Studies of dietary supplementation with pomegranate juice have shown protective effects against atherogenesis and atherosclerosis as well as reduction in serum angiotensin-converting enzyme activity with subsequent reduction in systolic blood pressure (25-27). Maternal dietary supplementation with pomegranate juice would result in protection against neonatal brain injury (28). So, in this work we used a well-characterized model of term H/I in adult male rats to investigate the neuroprotective capability of pomegranate seed hydroalcoholic extract in a treatment paradigm.

Materials and Methods

Animals: Eighty adult male albino rats of Wistar strain (250±20g, 3-4 months) obtained from Ahvaz Jundishapur University of Medical Sciences (AJUMS) laboratory animal center were used in this study. Animals were housed in standard cages under controlled room temperature (20±2 °C), humidity (55-60%) and light exposure conditions 12:12 h light-dark cycle (lighted on 07:00 am). All experiments carried out during the light phase of the cycle (8:00 am to 6:00 pm). Access to food and water were ad libitum except during the experiments. Animal handling and experimental procedures performed under observance of the University and Institutional legislation, controlled by the Local Ethics Committee for the Purpose of Control and Supervision of Experiments on Laboratory Animals. All efforts were made to minimize animal suffering, to reduce the number of animals used. Prior to the onset of behavioral testing, all rats were gentle handled for 5 days (daily 5 min). Animals were divided randomly into five groups, consisting of 10 animals in each:

- 1) Sham-operated (Sh.I) with manipulation of both common carotids arteries without occlusion.
- 2) Untreated Ischemic group with occlusion of bilateral common carotids arteries (I).
- 3) Ischemic rats received PGE (100mg/kg,orally) for 14 days (I.E100). 4) Ischemic rats received PGE (200mg/kg,orally) for 14 days (I.E200).
- 5) Ischemic rats received PGE (400mg/kg,orally) for14 days (I.E400).
- 6) Ischemic rats received PGE (800mg/kg,orally) for 14 days (I.E800).
- Sham treatted animals received same volume of normal saline as PGE vehicle (2ml/kg,orally) for 14 days (I.Veh).
- 8) Positive control group received the best effective dose of PGE (400mg/kg,orally) for 14 days (C.E400).

CCAO procedure:

Cechetti's method (2010) with little modification was used. In summary, rats were anesthetized with ketamine/xylazine (50/5mg/kg, i.p). A neck ventral midline incision was made and the common carotid arteries were then exposed and gently separated from the vagus nerve. Carotids were occluded with a one week interval between interventions, the right common carotid being the first to be assessed and the left one being occluded 1 week later. Sham-operated controls were under same surgical procedures without carotid artery ligation and occlusion (29).

PGE preparation:

Pomegranate fruits (Punica granatum L.) as large fruit with red barriers were purchased from Shahreza granatum gardens- Iran. Seeds removed from the fruits, air dried in shade for one week and milled to fine powder (electric mill, Panasonic Co. Japan). The seeds powder was macerated in 70% ethanol for 72 hours at room temperature. The ethanol extract evaporated (Rotary Ovaporator, Heidolph Co. Germany) to remove ethanol and PGE was obtained as a lyophilized powder (yield $17\pm2\%$).

Treatment:

Different doses of extract were administrated to each animal in separate groups via forced oral administration (i.g.) everyday 8:00–9:00 am for 2 weeks, starting on the 5 days post ischemic injury. Sham treated animals (I.Veh) were received same volume of normal saline for same period.

Passive avoidance task:

The apparatus used for evaluation the passive avoidance task was two-way shuttle box (Borj Sanaat Co. Iran), which consisted of two adjacent Plexiglas compartments of identical dimensions (27×14.5×14 cm). Light compartment was illuminated by a 5 W lamp mounted on its wall just below a movable transparent Plexiglas ceiling. For the experimental procedure, on the first day (adaptation period) each rat was allowed a 10 minutes adaptation period prior and free access to either the light or dark compartment of the box to avoidance training and after being placed in a shuttle-box. Following this adaptation period, on the second day (initial phase) rats were placed into the illuminated compartment and 30 seconds later the sliding door was raised. Upon entering the dark compartment the door was closed and a 1.5 mA constant-current shock was applied for 3 seconds. After 20 seconds the rat was removed from the dark compartment and placed into home cage. In order to test short-term, 24 hours after receiving foot shock, the rats were placed in illuminated chamber and 30 seconds later the sliding door was raised and the latency of entering the dark compartment (step-through latency) was recorded again, because the maximum time that considered in this procedure were 300 seconds (30-32).

Statistics

Data were expressed as mean \pm S.E.M. of values for initial latency and memory tests. Statistical analysis was performed by one-way ANOVA followed by LSD post hoc test. A P-value less than 0.05 were assumed to denote a significant difference and levels of significance are indicated by symbols: * or \$ P<0.05, ** or \$\$ P<0.01, *** or \$\$\$P<0.001.

Results

Data show that initial latency (s) for leaving rats from light to dark compartment of shuttle box before exposing them to any serious stimulus (electrical shock) was decreased significantly (P<0.001) in ischemic rats two weeks after 2CCAO when compared with sham ischemic group. Treatment of 2CCAO rats with oral administration of 100 mg/kg of PGSE for 14 days improved initial latency significantly when compared with Sh.I and I groups (P<0.001 for I.E100 vs. I and P<0.05 for I.E100 vs. Sh.I respectively), yet initial latency of I.E100 after 14 days treatment with PGSE was less than Sh.I groups. On the other hand, didn't change initial latency of ischemic animals after treatment with same volume of PGSE vehicle (normal saline). Also treatment the ischemic rats with each other separated doses of PGSE (200, 400 and 800 mg/kg, ig. for 14 days) improved significantly initial latency so that it didn't any difference with Sh.I group (P<0.001 for each one of I.E200, I.E400 or I.E800 vs. I group respectively) (figure 1). Data show that dose 400 mg/kg of PGSE was the lower with the same effect versus higher dose (800mg/kg) of extract, so this dose was administrated to healthy intact rats as positive group but PGSE couldn't affect initial latency significantly (figure 2). Short-term passive avoidance memory (24 hours after exposing the electrical shock to paws of rats) in all groups was evaluated with same producer while no shock delivered to animals. As shown in figures 3 this type of memory was impaired significantly in both no- treated ischemic and also in ischemic rats received PGSE vehicle (P<0.001 for I. and I.Veh. groups vs. Sh.I). In other hand, treatment of ischemic groups with each other doses of extract causes improving the memory significantly when compared with non treated ischemic rats (I group) (P<0.001 for all treated groups with PGSE vs. I) so that initial latency in treated ischemic groups reversed to control level and didn't differ with Sh.I



Figure 1. Mean \pm SEM of initial latency (s) just before exposing to electrical shock to paws as passive avoidance learning of sham ischemic (Sh.1), ischemic (1), I.E100, I.E200, I.E400, I.E800 and sham treated (I.Veh) received normal saline2 ml/14 days). Data were analyzed by one-way ANOVA followed by LSD post hoc test, symbols *P<0.05 and *** P<0.001for significant difference with Sh.I and \$\$\$ P<0.001 for significant difference with I groups respectively.



Figure 3. Mean \pm SEM of step-trough latency (s) (after 24h) as passive avoidance memory of sham ischemic (Sh.I), ischemic (I), I.E100, I.E200, I.E400, I.E800 and sham treated (I.Veh) received normal saline2 ml/14 days). Data were analyzed by one-way ANOVA followed by LSD post hoc test, symbols ** P<0.01 for significant difference with Sh.I and \$\$ P<0.01 for significant difference with I groups respectively

rats. Also these data showed that passive avoidance memory in healthy intact rats received dose 400 mg/kg PGSE that had the best effect on initial latency test, couldn't change the memory after 14 days administration of extract (figure 4).



Figure 2. Mean \pm SEM of initial latency (s) just before exposing to electrical shock to paws as passive avoidance learning of sham ischemic (Sh.I), ischemic (I), and healthy intact group received the best dose of PGSE (400 mg/kg/2 ml). Data were analyzed by one-way ANOVA followed by LSD post hoc test. *P<0.05 and *** P<0.001 for significant difference with Sh.I and \$\$\$ P<0.001 for significant difference with I groups respectively



Figure 4. Mean \pm SEM of step-trough latency (s) (after 24h) as passive avoidance memory of sham ischemic (Sh.I), ischemic (I), and healthy intact group received the best dose of PGSE (400 mg/kg/2 ml). Data were analyzed by one-way ANOVA followed by LSD post hoc test, symbols ** P<0.01 for significant difference with Sh.I and \$\$ P<0.01 01 for significant difference with I groups respectively

Discussion

According to our findings Learning and memory was impaired in hypoperfusion/ischemia rats. We have found that 14 days oral administration of different doses of PGSE can improve passive avoidance learning and memory in ischemic rats. Dose 400 mg/kg of PGSE was select as the best effective dose to significant improving of initial latency and short-term memory (24h) in ischemic groups. Also 14 days treatment with this dose changed neither initial latency nor short-term memory in healthy intact rats.

Interestingly, extracts from natural substances have the ability to protect neurons from ischemic damage (33-34). The extracts have several functions including antioxidant effects in the neuroprotection from ischemic insults (35).

Phytochemical investigation of ethanol extract for the presence of phenolic compounds, flavonoids, tannins, anthocyanins, sugars and saponins was also carried out. Phytochemical screening and measurement of reducing power revealed the CNS activity of ethanol extract of PGSE may be due to its antioxidative profile (36-37). Another study also showed that pomegranate (Punica granatum) seed extract contains several compounds such as linolenic acid, elagic acid, punic acid, alpha-eleostearic acid (AEA), flavonoids, polyphenols, ellagitannin (37% punicalagins) and other useful contents. Pomegranate seed linolenic acid isomers were evaluated as selective estrogen receptor modulators (SERMs). Punicic acid (PA) inhibited (IC-50) estrogen receptor (ER) alpha at 7.2 microM, ERbeta at 8.8 microM; AEA inhibited ERalpha/ERbeta at 6.5/7.8 microM. PA (not AEA) agonized ERalpha/ERbeta (EC-50) at 1.8/2 microM, antagonizing at 101/80 microM (38). About the ellagitannin in pomegranate, recently, potent anti-tumorigenic effects of pomegranate juice and extracts have been reported. In the other word, pomegranate has potential not only as a treatment but also as a preventative measure against certain types of cancer, including prostate (39). Another study via biochemical analysis revealed that pomegranate with highest antioxidant capacity was found in leaves followed by peel, pulp, and seed. Pomegranate seed had an average lipid content of 19.2% with punicic acid as the predominant fatty acid. Pomegranate seed has high contents of alpha-tocopherol (161.2-170.1 mg/100 g) and gamma-tocopherol (80.2-92.8 mg/100 g) (40).

In an investigation carried out by Turkish researcher during 2007, twenty different varieties of pomegranate (Punica granatum) from Turkey were analyzed for vitamin C level, lipid content, sterol determination, anthocyanin content, and elemental analysis (Ca, Mg, P, Fe, Na, and K studies). Vitamin C content range of 1,050-312 mg/100 g, oil content range of 2.41-3.73%, sterol content range of 5.78-8.43%, anthocyanin content range of 2,100-4,400 mg/L, potassium range of 250-1,200 ppm, calcium range of 35-326 ppm, magnesium range of 176-427 ppm, iron range of 21-46 ppm, sodium range of 35-76 ppm, and phosphorus range of 12-43 ppm were observed in these varieties (18-19). Although in the literature studied, we have found few specific references to effects of PGSE on brain damages due to H/I or degeneration. So, on base of results in our work, we like to suggest that PGSE with these compounds beside other useful contents such as ellagic acid, phytoestrogens, punicic acid could act as an antioxidant for scavenge the free radicals from brain regions that influence cognition especially hippocampus, striatum and cortex after H/I. PGSE had no toxicity after administration of different doses (100-800 mg/kg, ig) for 14 days in healthy and ischemic rats. Of course mortality of 30-35% in our rats occurred only during recovery from ischemia surgery in all ischemic groups (not cause by PGSE) and same range of mortality has early reported by others. The acute oral toxicity study revealed no significant findings at 2000 mg PSO/kg body weight. In the 28-day dietary toxicity study PSO was dosed at concentrations of 0, 10,000, 50,000 and 150,000 ppm, which resulted in a mean intake of 0-0, 825-847, 4269-4330 and 13,710-14,214 mg PSO/kg body weight per day in males-females, respectively (41-42).

During the period of ischemia large quantities of stimulatory amino acids are released and calcium overload, lead to increase in free radicals that is the signs of point that is called exitoxicity phase (43). Both a great production of free radicals and the deficiency or depletion of many antioxidant systems may reveal exacerbation of the oxidative cellular injury, while the supplementation of many

antioxidants generates diverse outcomes (44-45). Free radicals are highly reactive molecules that may be formed during various biochemical reactions in the cell. Many of these free radicals contain oxygen and are called ROS. Typically, the levels of ROS and other free radicals are controlled by various scavenger molecules, known as antioxidants, that are normally found within the cell and which eliminate free radicals. The antioxidant defense mechanisms include antioxidant enzymes like SOD, GPx and several non-enzymatic free radical scavengers (46). If ROS levels exceed the cell's ability to eliminate them or if the normal antioxidant levels within the cell are reduced due to a toxic agent such as alcohol, then oxidative stress can occur. This oxidative stress can cause damage to cellular components, such as membranes, DNA, and proteins (47). The brain is more vulnerable to oxidative stress than other organs due to its low antioxidant protection system and increased exposure of target molecules to reactive oxygen species. The nervous tissue has a high content of polyunsaturated fatty acids 5(48), which are easy targets to oxidative damage by free radicals due to the unsaturated bonds they contain (49). On the other hand, it has been revealed that brain structures supporting memory are uniquely sensitive to oxidative stress due to their elevated demand for oxygen (50-51). It has showed that hippocampus is a brain area particularly susceptible to ischemia-induced oxidative stress. However, its antioxidative activity in central nervous system and its effects on spatial memory deficits induced by ischemia have not been scientifically documented so far (52). Behavioral studies in animals have demonstrated that hippocampal damage can produce learning and memory impairments (16, 53), particularly on tasks that involve place learning (54). In a study, it has demonstrated that supplementing the mouse maternal diet with pomegranate juice, a food highly enriched in polyphenols, in the per partum period provides significant neuroprotection from hypoxic-ischemic injury to neonatal mouse. It has been shown that modulation of nitric oxide (NO) availability is an important determinant of ischemic stroke risk (55). Thus optimal NO/ROS balance in the brain seems to be a crucial parameter in the prevention of brain damage including ischemic stroke and neurodegenerative

diseases as well. The neuroprotective effects of many polyphenols presente in PGSE rely on their ability to permeate brain barrier and here directly scavenge pathological concentration of reactive oxygen and nitrogen species and chelate transition metal ions (56). Different polyphenolic compounds were shown to have scavenging activity and the ability to activate key antioxidant enzymes in the brain and thus breaking the vicious cycle of oxidative stress and tissue damage (57-58). There is a growing interest in the potential of natural polyphenols to improve memory, learning and general cognitive ability. Recent evidence has indicated that flavonoids may exert especially powerful actions on mammalian cognition and may reverse age-related declines (18, 59).

In conclusion, PGSE improves memory deficiency due to H/I in rats because its contents, such as vitamines, phytoestrogens, ellagic acid, punicic acid, polyphenols and flavonoids act as antioxidant and remove free radicals in damaged brain tissue after H/I. Overally, the exact mechanisms the effect of PGSE on cognition needs more attentions for investigations.

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References

- 1. Aquilano K, Baldelli S, Rotilio G, Ciriolo MR. Role of nitric oxide synthases in Parkinson's disease: a review on the antioxidant and anti-inflammatory activity of polyphenols. Neurochem Res. 2008 Dec; 33(12):2416-26.
- Aviram M, Dornfeld L, Kaplan M, Coleman R, Gaitini D, Nitecki S, et al. Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. Drugs Exp Clin Res. 2002;28(2-3):49-62.

- 3. Aviram M, Dornfeld L. Pomegranate juice consumption inhibits serum angiotensin converting enzyme activity and reduces systolic blood pressure. Atherosclerosis. 2001 Sep;158(1):195-8.
- Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, et al. Pomegranate juice consumption reduces oxidative stress, atherogenic modifications to LDL, and platelet aggregation: studies in humans and in atherosclerotic apolipoprotein E-deficient mice. Am J Clin Nutr. 2000 May; 71(5): 1062-76.
- Ben Nasr C, Ayed N, Metche M. Quantitative determination of the polyphenolic content of pomegranate peel. ZLebensm Unters Forsch. 1996 Oct; 203(4):374-8.
- Banerjee AK, Mandal A, Chanda D, Chakraborti S. Oxidant, antioxidant and physical exercise. Mol Cell Biochem. 2003 Nov;253(1-2):307-12.
- 7. Block F. Global ischemia and behavioural deficits. Prog Neurobiol. 1999 Jun; 58(3): 279-95.
- Cechetti F, Worm PV, Pereira LO, Siqueira IR, C AN. The modified 2VO ischemia protocol causes cognitive impairment similar to that induced by the standard method, but with a better survival rate. Braz J Med Biol Res. 2010 Dec;43(12):1178-83.
- 9. Choi DW, Rothman SM. The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. Annu Rev Neurosci. 1990;13:171-82.
- Chun HS, Kim JM, Choi EH, Chang N. Neuroprotective effects of several korean medicinal plants traditionally used for stroke remedy. J Med Food. 2008 Jun;11(2):246-51.
- 11. Colbourne F, Li H, Buchan AM, Clemens JA. Continuing postischemic neuronal death in CA1: influence of ischemia duration and cytoprotective doses of NBQX and SNX-111 in rats. Stroke. 1999 Mar;30(3):662-8.
- 12. Collingridge GL, Kehl SJ, McLennan H. The antagonism of amino acid-induced excitations of rat hippocampal CA1 neurones in vitro. J Physiol. 1983 Jan; 334:19-31.
- 13. Esposito E, Rotilio D, Di Matteo V, Di Giulio C, Cacchio M, Algeri S. A review of specific dietary antioxidants and the effects on biochemical mechanisms related to neurodegenerative processes. Neurobiol Aging. 2002 Sep-Oct; 23(5):719-35.
- 14. Floyd RA. Antioxidants, oxidative stress, and degenerative neurological disorders. Proc Soc Exp Biol Med. 1999 Dec;222(3):236-45.
- 15. Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem. 2000 Oct;48(10):4581-9.

- Greenamyre JT, Olson JM, Penney JB, Jr., Young AB. Autoradiographic characterization of N-methyl-D-aspartate-, quisqualate- and kainate-sensitive glutamate binding sites. J Pharmacol Exp Ther. 1985 Apr;233(1):254-63.
- Hashimoto T, Yonetani M, Nakamura H. Selective brain hypothermia protects against hypoxic-ischemic injury in newborn rats by reducing hydroxyl radical production. Kobe J Med Sci. 2003;49(3-4):83-91.
- Pilsakova L, Riecansky I, Jagla F. The physiological actions of isoflavone phytoestrogens. Physiol Res. 2010;59(5):651-64.
- 19. Dumlu MU, Gurkan E. Elemental and nutritional analysis of Punica granatum from Turkey. J Med Food. 2007 Jun;10(2):392-5.
- 20. Ito U, Spatz M, Walker JT, Jr., Klatzo I. Experimental cerebral ischemia in mongolian gerbils. I. Light microscopic observations. Acta Neuropathol. 1975 Aug 27;32(3):209-23.
- 21. Jee YS, Ko IG, Sung YH, Lee JW, Kim YS, Kim SE, et al. Effects of treadmill exercise on memory and c-Fos expression in the hippocampus of the rats with intracerebroventricular injection of streptozotocin. Neurosci Lett. 2008 Oct 10;443(3):188-92.
- 22. Takeda A, Tamano H, Tochigi M, Oku N. Zinc homeostasis in the hippocampus of zinc-deficient young adult rats. Neurochem Int. 2005 Feb;46(3):221-5.
- 23. Kim ND, Mehta R, Yu W, Neeman I, Livney T, Amichay A, et al. Chemopreventive and adjuvant therapeutic potential of pomegranate (Punica granatum) for human breast cancer. Breast Cancer Res Treat. 2002 Feb;71(3):203-17.
- 24. Kaplan M, Hayek T, Raz A, Coleman R, Dornfeld L, Vaya J, et al. Pomegranate juice supplementation to atherosclerotic mice reduces macrophage lipid peroxidation, cellular cholesterol accumulation and development of atherosclerosis. J Nutr. 2001 Aug;131(8):2082-9.
- 25. Kostrzewa RM, Segura-Aguilar J. Novel mechanisms and approaches in the study of neurodegeneration and neuroprotection. a review. Neurotox Res. 2003;5(6):375-83.
- 26. Kirino T. Delayed neuronal death. Neuropathology. 2000 Sep;20 Suppl:S95-7.
- 27. Kirino T. Delayed neuronal death in the gerbil hippocampus following ischemia. Brain Res. 1982 May 6;239(1):57-69.
- 28. Lau FC, Shukitt-Hale B, Joseph JA. The beneficial effects of fruit polyphenols on brain aging. Neurobiol Aging. 2005 Dec; 26 Suppl 1:128-32.

- 29. Leeuwenburgh C, Heinecke JW. Oxidative stress and antioxidants in exercise. Curr Med Chem. 2001 Jun;8(7):829-38.
- 30. Lipton SA, Rosenberg PA. Excitatory amino acids as a final common pathway for neurologic disorders. N Engl J Med. 1994 Mar 3;330(9):613-22.
- 31. Mahut H, Zola-Morgan S, Moss M. Hippocampal resections impair associative learning and recognition memory in the monkey. J Neurosci. 1982 Sep;2(9):1214-20.
- 32. Levy DE, Caronna JJ, Singer BH, Lapinski RH, Frydman H, Plum F. Predicting outcome from hypoxic-ischemic coma. JAMA. 1985 Mar 8;253(10):1420-6.
- 33. Meyer FB, Sundt TM, Jr., Yanagihara T, Anderson RE. Focal cerebral ischemia: pathophysiologic mechanisms and rationale for future avenues of treatment. Mayo Clin Proc. 1987 Jan;62(1):35-55.
- 34. McCarty MF. Up-regulation of endothelial nitric oxide activity as a central strategy for prevention of ischemic stroke - just say NO to stroke! Med Hypotheses. 2000 Nov;55(5):386-403.
- 35. Evans PH. Free radicals in brain metabolism and pathology. Br Med Bull. 1993 Jul;49(3):577-87.
- 36. Nazam Ansari M, Bhandari U, Islam F, Tripathi CD. Evaluation of antioxidant and neuroprotective effect of ethanolic extract of Embelia ribes Burm in focal cerebral ischemia/reperfusion-induced oxidative stress in rats. Fundam Clin Pharmacol. 2008 Jun;22(3):305-14.
- 37. Kumar S, Maheshwari KK, Singh V. Central nervous system activity of acute administration of ethanol extract of Punica granatum L. seeds in mice. Indian J Exp Biol. 2008 Dec;46(12):811-6.
- 38. O'Keefe J, Conway DH. Hippocampal place units in the freely moving rat: why they fire where they fire. Exp Brain Res. 1978 Apr 14;31(4):573-90.
- Petito CK, Feldmann E, Pulsinelli WA, Plum F. Delayed hippocampal damage in humans following cardiorespiratory arrest. Neurology. 1987 Aug; 37(8):1281-6.
- 40. Pellegrini-Giampietro DE, Cherici G, Alesiani M, Carla V, Moroni F. Excitatory amino acid release from rat hippocampal slices as a consequence of free-radical formation. J Neurochem. 1988 Dec;51(6):1960-3.
- 41. Pulsinelli WA, Brierley JB, Plum F. Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol. 1982 May;11(5):491-8.
- 42. Meerts IA, Verspeek-Rip CM, Buskens CA, Keizer HG, Bassaganya-Riera J, Jouni ZE, et al. Toxicological evaluation of pomegranate seed oil. Food Chem Toxicol. 2009 Jun;47(6):1085-92.

- 43. Qian ZJ, Jung WK, Kim SK. Free radical scavenging activity of a novel antioxidative peptide purified from hydrolysate of bullfrog skin, Rana catesbeiana Shaw. Bioresour Technol. 2008 Apr;99(6):1690-8.
- 44. Reiter RJ. Oxidative processes and antioxidative defense mechanisms in the aging brain. FASEB J. 1995 Apr;9(7):526-33.
- 45. Renis M, Calabrese V, Russo A, Calderone A, Barcellona ML, Rizza V. Nuclear DNA strand breaks during ethanol-induced oxidative stress in rat brain. FEBS Lett. 1996 Jul 22;390(2):153-6.
- 46. Murray L, Stein A. The effects of postnatal depression on the infant. Baillieres Clin Obstet Gynaecol. 1989 Dec;3(4):921-33.
- 47. Simon RP, Swan JH, Griffiths T, Meldrum BS. Blockade of N-methyl-D-aspartate receptors may protect against ischemic damage in the brain. Science. 1984 Nov 16;226(4676):850-2.
- 48. Sun AY, Simonyi A, Sun GY. The "French Paradox" and beyond: neuroprotective effects of polyphenols. Free Radic Biol Med. 2002 Feb 15;32(4):314-8.
- 49. Taati M, Moghadasi M, Dezfoulian O, Asadian P, Kheradmand A, Abbasi M, et al. The effect of ghrelin pretreatment on epididymal sperm quality and tissue antioxidant enzyme activities after testicular ischemia/reperfusion in rats. J Physiol Biochem. 2012 Mar;68(1):91-7.
- 50. Urso ML, Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. Toxicology. 2003 Jul 15;189(1-2):41-54.
- 51. Vannucci RC, Vannucci SJ. A model of perinatal hypoxic-ischemic brain damage. Ann N Y Acad Sci. 1997 Dec 19;835:234-49.
- 52. Wickens AP. Ageing and the free radical theory. Respir Physiol. 2001 Nov 15;128(3):379-91.
- 53. Wigstrom H, Gustafsson B, Huang YY. Mode of action of excitatory amino acid receptor antagonists on hippocampal long-lasting potentiation. Neuroscience. 1986 Apr;17(4):1105-15.
- 54. Yoo KY, Li H, Hwang IK, Choi JH, Lee CH, Kwon DY, et al. Zizyphus attenuates ischemic damage in the gerbil hippocampus via its antioxidant effect. J Med Food. 2010 Jun;13(3):557-63.
- 55. Zola-Morgan S, Squire LR. Memory impairment in monkeys following lesions limited to the hippocampus. Behav Neurosci. 1986 Apr;100(2):155-60.
- 56. Tran HN, Bae SY, Song BH, Lee BH, Bae YS, Kim YH, et al. Pomegranate (Punica granatum) seed linolenic acid isomers: concentration-dependent modulation of estrogen receptor activity. Endocr Res. 2010 Jan; 35(1):1-16.

- 57. Koyama S, Cobb LJ, Mehta HH, Seeram NP, Heber D, Pantuck AJ, et al. Pomegranate extract induces apoptosis in human prostate cancer cells by modulation of the IGF-IGFBP axis. Growth Horm IGF Res. 2010 Feb;20(1):55-62.
- 58. Pande G, Akoh CC. Antioxidant capacity and lipid characterization of six Georgia-grown pomegranate cultivars. J Agric Food Chem. 2009 Oct 28;57(20):9427-36.
- 59. Sarkaki A, Rafieirad M, Hossini SE, Farbood Y, Mansouri SMT, Motamedi F. Cognitive deficiency induced by cerebral hypoperfusion/ischemia improves by exercise and grape seed extract. HealthMED. [Original]. 2012 1012;6(4):7.

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Emergence of multidrug resistance phenotype in clinical isolates of Clostridium difficile as a risk factor in occurrence of antibiotic associated diarrhea

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Abstract

Clostridium difficile is a Gram-positive spore forming anaerobic bacteria which is considered as the main etiological agent of hospital acquired diarrhea. The aim of this study was to determine the drug resistance profiles against conventional administrative drugs like metronidazole, amikacin, ceftazidime, imipenem and ciprofloxacin among clinical isolates of C.difficile in patients with AAD. C. difficile isolates which had been obtained from fecal samples of suspicious patients to AAD were cultured on specific medium and confirmed with polymerase chain reaction (PCR) amplification of cdd3 gene. Susceptibility of the isolates was determined against five different antibiotics by disc diffusion method. Our findings from this study showed that the resistant to ceftazidime, amikacin, imipenem, and ciprofloxacin were 91(96%), 89(94%), 81(85%) and 65(68%) respectively. No resistant isolate to metronidazole were found. According to the prescribed antibiotic regimes against AAD in Iran and antibiotic resistance pattern, it seems that, metronidazole still can be effective for removing the infection of C. difficile in Iran.

Keywords: Clostridium difficile, antibiotic resistance, disc diffusion method.

Introduction

The most repeated side effects of antibiotic therapy are diarrheas. The signs may vary from some abdominal inconvenience to severe diarrhea and colitis (1). Antibiotic associated diarrhea (AAD) can cause in different manners. Destruction of enteric normal flora caused by antibiotics therapy during hospitalization will lead to overgrowth of resistant pathogens, and functional disorders of the intestinal carbohydrate and bile acid metabolism which will result to diarrhea (1). One of these pathogenic bacteria is Clostridium difficile an anaerobic, spore-forming, Gram-positive bacterium. The organism produces at least two toxins: toxin A (enterotoxin) and toxin B (cytotoxin) (2). Toxin B of C. difficile is reported to be the causal agent of almost 20 per cent of AAD and of nearly all cases of pseudomembranous colitis, the most severe manifestation of AAD (1). The occurrence of C. difficile diarrhea has risen a lot in recent years because of the repeated use of broad spectrum antibiotics (3, 4). However plenty infants and hospitalized patients can be colonized with C. difficile without any signs of infection (5, 6). In hospitals and nursing homes where patients repeated receive antibiotics C. difficile infection is highly prevalent and shows one of the most common hospital infections (7, 8, 9, 10). Conventional treatment regimens for AAD include administration of metronidazole and oral vancomycin for 10 to 14 days (11). However, reduced sensitivity of C. difficile isolates to metronidazole and increased refractories of the disease have been reported. Metronidazole is still considered as the best medicine for treating the infections caused by the C. difficile (12, 13). There are few data about antimicrobial resistance profiles of C. difficile clinical isolates in Iran (14). In current study we aimed to evaluate the drug resistance profiles against conventional

administrative drugs like metronidazole, amikacin, ceftazidime, imipenem and ciprofloxacin among clinical isolates of C. difficile in patients with AAD.

Materials and methods

Bacterial strains

Ninety five isolates of C. difficile had been collected from fecal samples of suspicious patients to AAD in a referral laboratory of C. difficile at Taleghani Hospital in Tehran, Iran (RCGLD). All demographic data about patients, including age, gender, antibiotic treatment and medications' history were collected in a standard questionnaire.

Culture and isolation

All fecal specimens from patients suspected of having AAD cultured on proper culture media after their treatments by two following methods. At first method, small amount of stool samples were mixed with 1 ml of 5% Yeast extract broth and directly inoculated onto C. difficile medium (Mast, London, United Kingdom) supplemented with 7% horse blood and C. difficile selective supplement consisting of D-cycloserine (250 mg/ liter), cefoxitin (8 mg/liter), and lysozyme (5 mg/liter) (Mast, United State of America). The cultured plates were incubated at 37 °C for at least 48-72 h under anaerobic conditions (80% N2; 10% CO2 and 10% H2), had been produced by anaerobic generation system Anoxomat - Mart (Microbiology, Poland). At the second method, the stool samples were mixed completely with 1 ml of methanol (alcohol-shock procedure) for 1 to 2 minutes before inoculation on the C. difficile medium. The cultures results were followed up to one week. Isolates with characteristic colony morphologies and Gram staining for C. difficile were followed for further identification by specific primers. Subcultures of the confirmed isolates were stored in cooked meat broth (Himedia, India) at 4°C (15).

DNA extraction and molecular identification

C. difficile colonies were suspended in 500 μ l of distilled water in 1.5 ml sterile microtube and vortexed. The cells were washed with distilled water by centrifugation for 10 min at 13000 g. The

pellets were mixed with 100 µl of distilled water and incubated in a boiling bath for 10 min. Supernatants, which contained DNA, were transferred to new 1.5 ml sterile micro tubes after centrifugation for 10 min at 13000 g. The DNA samples were stored at -20 °C until use. For identification of the suspected colonies PCR for detection of cdd3 gene was done using specific primer pairs Tim6: 5'- TCCAAT ATAATAAATTAGCATTCC -3' and Struppi6: 5'- GGCTATTACACGTAATCCAGA-TA-3', which amplify 622-bp fragment of the gene (13). The PCR was performed in a final volume of 25 µl containing 2.5 µl of 10 X PCR buffer, 0.5 µl of each primer (20 picomol), 1.5 µl of MgCl2 (50mM), 0.5 µl of each deoxyribonucleotide triphosphate (dNTP) (10mM), 0.5 U of TaqDNA polymerase (5U/ml) and 1 µl of the DNA sample (400 mg/µl). It was performed in a thermocycler (AG 22331; Eppendorf, Hamburg, Germany) under the following conditions: initial denaturation for 5 min at 95°C followed by 40 cycles of 95°C for 1 min, 58°C for 1 min, 72°C for 1 min and a final extension at 72 °C for 10 min. Electrophoresis on 1.2 % agarose was followed according to standard procedures.

Determination of antibiotic susceptibility by the disc diffusion method

To screen antibiotic susceptibility of the isolates, their initial sensivity was analyzed against metronidazole, amikacin, imipenem, ceftazidime and ciprofloxacin by Kirby-Bauer disc diffusion method. The isolates were subcultured from cooked meat broth into C. difficile medium supplemented with 7% horse blood. Colonies of C. difficile were suspended in sterile saline buffer (no. 1 McFarland standard) and swabbed on Brucella agar (Merck Co, Germany) supplemented with 7% defibrinated sheep blood. Standard discs of metronidazole (5 mg; Mast, United Kingdom), amikacin (30 mg; Padtanteb, Iran), imipenem (10 mg; Padtanteb ,Iran), ceftazidim (30 mg; Padtanteb,Iran) and ciprofloxacin (5 mg; Padtanteb, Iran) were used in the assay. The plates were incubated in anaerobic jars at 37 °C for 24-48 h under anaerobic atmosphere (80% N2; 10% CO2 and 10% H2). Interpretation of the results was done according to use breakpoints by other researchers in this field (16).
Results

Here we examined a population (n = 95) of Iranian C. difficile isolates, which were isolated from Taleghani hospital in Tehran.

To screen antibiotic susceptibility of the isolates, their initial sensitivity was analyzed against metronidazole, amikacin, imipenem, ceftazidime and ciprofloxacin by Kirby-Bauer disc diffusion method. All 95 C. difficile isolates with criterion as Gram positive, bacilli-shaped spore forming bacteria, characteristic colony morphologies, and cdd3 positive PCR reaction were selected for antibiotic susceptibility test. Assessed sensitivity of C. difficile isolates against the noted antibiotics by the disk diffusion method proposed existence of resistance isolates in high frequency as follow: ceftazidime (96%), amikacin (94%), imipenem (85%) and ciprofloxacin (68%). In the case of metronidazole an inhibition zone ranging from 30 to 45 mm was seen for all the isolates, which was considered as an initial indicator of susceptibility to this antibiotic (diagram1). The antibiogram patterns of isolates showed a high percentage of multi drug resistant (MDR) phenotype among the tested C. difficile isolates. Twelve percent of the isolates were resistant to at least two drugs and twenty-three percent of them showed resistance to at least three drugs. The predominant pattern among these isolates were included resistance to ceftazidime, amikacin, imipenem and ciprofloxacin (57%), which was common among 54 isolates. In this study there was not any significant relation between antibiotic resistance and sex, age and patients outcomes.



Diagram 1. Antimicrobial susceptibility to five different antibiotics by Disc diffusion method for 95C. difficile isolates

Discussion

It has been shown that using antibiotics is the most important factor in the acquisition of AAD. These antibiotics include broad spectrum beta lactams, cephalosporins, clindamycin and the new fluoroquinolones. The best strategy for treatment of AAD includes stopping the causative antibiotic and directly beginning treatment with metronidazole or vancomycin. Manifestation of resistant isolates of C. difficile to metronidazole and vancomycin is now a reason of great concern worldwide that can limit this treatment strategy. In spite of high frequency of C. difficile infection and antibiotic usage in Iran, few data are present to describe the antibiotic resistance pattern among these isolates. Metronidazole is a nitroimidazole antibiotic medication used particularly for anaerobic bacteria and protozoa (17). It is the drug of choice for first episodes of mild-to-moderate C. difficile infection (18). Resistance against metronidazole varies in different countries and in different years. For example in 2002 in a study on 415 strains, all of them showed sensitive phenotype to metronidazole in Spain (19). In 2006, all historic isolates were susceptible to metronidazole, in Canada (20). In 2007, similar results were obtained in south-east Scotland (21). In 2010, it was shown that from 606 C. difficile isolates, all were inhibited by a concentration of 2 mg/L of metronidazole in Sweden, (22). All the isolates in our study were inhibited by standard discs of metronidazole (5 mg; Mast, United Kingdom) and no isolates were found to be metronidazole resistant, similarly. Fluorquinolones, including ciprofloxacin, are a large family of broad-spectrum antibiotics and are widely associated with C. difficile infections. Ciprofloxacin is an efficient antibiotic against main enteric pathogens, including Campylobacter spp., Salmonella spp., and Shigella spp. (23). It is used also in treatment of the skin, lungs, airways, bones, and joints infections. Our results from this study showed that more than half (68%) of the isolates in Iran were resistant to this antibiotic. The range of resistance to ciprofloxacin in Iran was similar to this range in other countries. For example, the study had been conducted in 2006 in Ireland, showed that all isolates were resistant to ciprofloxacin (24). Another study had been conducted

in the Netherlands in 2010, also showed that all isolates were resistant to ciprofloxacin (25). It is possible that these increased levels of resistance to ciprofloxacin in clinical isolates of C. difficile in Iran and other countries be a result of inappropriate usage of this drug for diverse disease types. Our experience from this study showed that almost 96% of isolates in Iran were resistant to ceftazidime which is a third-generation cephalosporin antibiotic. Our results from this study were similar to another study from the United States in 2011 (26). Emerging ceftazidime resistance isolates of C. difficile in Iran may occurred due to widely usage of this antibiotic in most severe infections which is a serious threat to human health. Frequency of β-lactam resistance isolates was detected as 85%. The ranges of resistance to imipenem in Iran according to this study, was similar to these ranges in other countries. For example high level of imipenem resistance (97% of 606 isolates) was observed in Sweden (1993-2008) (27). Emergence and colonization of imipenem resistant C. difficile isolates may have resulted from bowel colonization of other resistant bacteria and transmission of the genetic markers by transformation or a conjugation-like mechanism (28). About amikacin which is an aminoglycoside antibiotic and usually use for treatment of different types of bacterial infections, the resistance rate was detected as 94% that indicated high level of resistance to this drug in Iran .Results of this study, collectively showed high frequency of antibiotic resistance phenotype among clinical isolates of C. difficile in Iran. Since the medical community has great concern about emergences of newly resistant bacteria responsible for human infections, high frequency of resistance rates to conventional antibiotics in these clinical isolates could be an alarming news in this context. However, finding of this in vitro study confirmed effectiveness of metronidazole for these isolates in Iran. Additional studies can help us to prepare detailed information about levels of these resistances and their importance in prediction of the disease outcome.

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References

- 1. Wistrom J, Norrby SR, Myhre EB, Eriksson S, GranstronG,Legergrea, et al. Frequency of antibioticassociated diarrhea in 2462 antibiotic-treated hospitalized patients: a prospectivestudy. J AntimicrobChemother2001; 47: 43-50.
- Bartlett JG. Clostridium difficile: history of its role as an entericpathogen and the current state of knowledge about theorganism. Clin Infect Dis 1994; 18(Suppl 4): S265-272
- 3. Dallal RM, Harbrecht BG, Boujoukas AJ, Sirio CA, FarkasLM, Lee KK, et al. Fulminant Clostridium difficile: anunderappreciated and increasing cause of death and complications. Ann Surg2002; 235 : 363-72.
- 4. Kyne L, Hamel MB, Polavaram R, Kelly CP. Health care costsand mortality associated with nosocomial diarrhea due toClostridium difficile. Clin Infect Dis 2002; 34 : 346-53.
- Larson HE, Barclay FE, Honour P, Hill ID. Epidemiology of Clostridium difficilein infants. J Infect Dis. 1982;146(6):727-733.
- 6. Al-Jumaili IJ, Shibley M, Lishman AH, Record CO. Incidence and origin of Clostridium difficilein neonates. J ClinMicrobiol1984;19(1):77-78.
- Alfa, M. J., T. Du, and G. Beda. 1998. Survey of incidence of Clostridiumdifficileinfection in Canadian hospitals and diagnostic approaches. J. Clin.Microbiol. 36:2076–2080.
- 8. Anton, P. M., et al. 2004. Rifalazil treats and prevents relapse of Clostridiumdifficile-associated diarrhea in hamsters. Antimicrob. Agents Chemother:48:3975–3979.
- 9. Kelly, C. P., C. Pothoulakis, and J. T. LaMont. 1994. Clostridium difficilecolitis. N. Engl. J. Med. 330:257– 262.
- Lai, K. K., Z. S. Melvin, M. J. Menard, H. R. Kotilainen, and S. Baker. 1997.Clostridium difficileassociated diarrhea: epidemiology, risk factors, and infectioncontrol. Infect. Control Hosp. Epidemiol. 18:628–632.
- 11. James Yoo, Amy Lee Lightner, Clostridium difficileInfections:What Every Clinician Should Know. The Permanente Journal/ Summer 2010/ Volume 14 No. 2
- 12. Kelly CP, LaMont JT. Clostridium difficile—more difficult than ever. N EnglJ Med 2008;359:1932–40.

- 13. MazenIssa, Ashwin N. Ananthakrishnan, and David G. Binion. Clostridium difficile and Inflammatory Bowel Disease.Inflamm Bowel Dis, Volume 14, Number 10, October 2008
- 14. Akhi MT, Pirzade T, Naghili B and Gojazade M. Antimicrobial susceptibility of Clostridium difficileisolated from different sources of Imam Reza Hospital, Tabriz. African Journal of Microbiology Research . 2011. Vol. 5(19) pp. 2946-2949.
- Brown, R., Collee, J. G. &Poxton, I. R. (1996). In Mackie and McCartney Practical Medical Microbiology, 14th edn, pp. 507–511.Edited by J. G.Collee, A. G. Fraser, B. P. Marmion& A. Simmons. Edinburgh:Churchill Livingstone.
- 16. European Committee on Antimicrobial Susceptibility TestingBreakpoint tables for interpretation of MICs and zone diametersVersion 2.0, valid from 2012-01-01
- 17. Metronidazole monograph". drugs.com. http:// www.drugs.com/monograph/metronidazole.html
- Cohen, S. H.; Gerding, D. N.; Johnson, S.; Kelly, C. P.; Loo, V. G.; McDonald, L. C.; Pepin, J.; Wilcox, M. H. et al. (2010). "Clinical Practice Guidelines for Clostridium difficile Infection in Adults: 2010 Update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of America (IDSA)". Infection Control and Hospital Epidemiology 31 (5): 431–455.
- 19. Pelaez, T., Alcala, L., Alonso, R., Rodriguez-Creixems, M., Garcia-Lechuz, J. M. &Bouza, E. (2002). Reassessment of Clostridium difficile susceptibility to metronidazole and vancomycin. Antimicrob Agents Chemother 46, 1647–1650.
- Bourgault Anne-Marie, Franc, oisLamothe, Loo Vivian G., Poirier Louise, and the CDAD-CSI Study Group. (2006). In Vitro Susceptibility of Clostridium difficileClinical Isolates from a Multi-Institutional Outbreak in Southern Que'bec, Canada. ANTI-MICROBIAL AGENTS AND CHEMOTHERAPY, p. 3473–3475.
- MutluEsvet, Wroe Allison J., Sanchez-Hurtado Karla, Brazier Jon S.andPoxton Ian R.(2007). Molecular characterization and antimicrobial susceptibility patterns of Clostridium difficile strains isolated from hospitals in south-east Scotland. Journal of Medical Microbiology, 56, 921–929.
- 22. Noren .T, Alriksson. I, kerlund. T. A ,Burman. L. G. and M. Unemo.(2010). In vitro susceptibility to 17 antimicrobials of clinical Clostridium difficile isolates collected in 1993–2007 in Sweden. ClinMicrobiol Infect; 16: 1104–1110.

- 23. Sara Sayadi, Mojtaba Darboue, Hosein Dabiri, Leila Shokrzadeh, Tabasom Mirzaee, Masoud Alebouyeh, Dariush Mirsatari, Homayoun Zojaji, Ehsan Nazemalhoseini, Mohammad Reza Zali.(2011). Study of antibiotic resistant H. pylori isolated from Iranian patients during 2009-2010. HealthMED -Volume 5; Number 6 - Suppl. 1:1970-1976.
- 24. gyrAMutations in FluoroquinoloneresistantClostridium difficilePCR-027. Emerging Infectious Diseases • Vol. 13, No. 3, March 2007.
- 25. D. Wultańska A. Banaszkiewicz A. Radzikowski P. Obuch-Woszczatyński G. Mlynarczyk J. S. Brazier H. Pituch A. van Belkum.(2010). Clostridium difficile infection in Polish pediatric outpatients with inflammatory bowel disease.Eur J ClinMicrobiol Infect Dis .DOI 10.1007/s10096-010-0997-9.
- 26. Michelle M. Nerandzic and Curtis J. Donskey. (2011). Effect of Ceftobiprole Treatment on Growth of and Toxin Production by Clostridium difficilein Cecal Contents of Mice. ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, p. 2174–2177.
- Norén T., Alriksson I., Andersson J., Unemo M. (2010). Heteroresistance to imipenem observed for clinical Clostridium difficile isolates using Etest susceptibility testing. 20th European Congress of Clinical Microbiology and Infectious Diseases. Vienna, Austria, 10 - 13 April 2010.
- 28. Leila Shokrzadeh1, Fereshteh Jafari1, Hossein Dabiri1(2010) Antibiotic Susceptibility Profile of Helicobacter pylori isolated from the Dyspepsia Patients in Tehran, Iran. Saudian journal of gasteroenterology

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Prevalence of overweight and obesity in northern Iran: an individual patient data meta-analysis

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Abstract

Introduction: Obesity is a universal problem involving about 300,000,000 people in the world and could lead to increase in mortality and reduction in life expectancy. In this study carried out an individual patient data(IPD)meta-analysis on data of the National STEPS Project.

Methods: The data of this study was part of the STEPS Study in Mazandaran province (northern Iran) which was conducted annually during 2005-2009. Sample size was 7759 subjects. Data entry were in Epi6 software and analyses were with stata 11 software.

Result: Standardized mean weight of the total sample was 70.06 kg, mean of height was 164.25 cm, mean waist circumference was 87.55 cm and mean of body mass index was 26.03 (males: 24.98, females: 27.11, p<0.0001). 53.7 percent (52.3-55.1) of total subjects had BMI more than 25 (overweight or obesity), while 20.8% (19.6-22.1) of them were obese(BMI>30).

Conclusion: Present study showed that prevalence of obesity and overweight is very high in this region of Iran. Therefore it needs that educational and research centres and health providers make suitable policies and strategies in order to prevent this problem.

Keywords: Obesity, overweight, BMI, Iran, prevalence.

Introduction

Obesity is a multifactorial disorder and one of the main problems in developed and developing countries which is important in health because of its high and growing prevalence (1-4). Obesity is considered as the unsuitable outcome of change in life style (5-8) and might lead to early disability and jobless as a consequence of its adverse complications(9-10). Overweight status beside other risk factors such as hypertension, smoking and hypercholesterolemia is an important factor for developing cardiovascular disorders, diabetes mellitus type 2 and some types of malignancies (11). Population based studies have shown that common none-communicable disorders are the main outcome of these risk factors (12) and obesity is the first cause for deteriorating of these illnesses(13). Obesity also is a universal problem involving about 300,000,000 people in the world and could lead to increase in mortality and reduction in life expectancy(14-15). Results of different studies, estimated the prevalence of obesity from 15 to 60% in various parts of the world(16).

Based on World Health Organization reports, about 16 millions of more than 15 years-old people had overweight status in 2005 and this prevalence is expected to increase up to 2.3 million overweight and 700 millions obese by 2015 (17-20). The prevalence of obesity and overweight in the Middle East in males and females were 31.4% and 52.4% respectively which has led to about 150,000 deaths per year(21).

In Iran, 70 percent of deaths wad due to chronic diseases in 2002 and overweight and obesity were the main cause. Based on WHO reports, in 2005, the prevalence of overweight status in males and females was 54% and 70% respectively and was predicted to increase to 74% in Iranian women during 2005-2015 but remains constant in men(22-23). In a systematic review in Iran, The prevalence of obesity was about 5.5% in age group under 18 and was 21.5% in the older ages(24). Considering the controversies in different relevant studies and

progressive prevalence of obesity in the world and Iran and remarkable changes have been occurred in the life styles, Investigation and monitoring of the situation and pattern of the body mean index (BMI)and detecting relevant risk factors of non communicable disorders is crucial and its findings could be used in designing interventions and programs for addressing relevant problems. Therefore a comprehensive study in Mazandaran (Northern Iran) was conducted to establish a useful data bank consisting important information about none communicable disease.

Unfortunately, almost all of the previous studies in this issue are either primary studies in restricted regions or systematic reviews and meta-analyses relying on aggregated results of original studies since there were no access to data other than those reported by the original authors. Therefore some errors such as aggregate biases might distort the results. On the other hand, meta-analyses of individual patient data can overcome some of the limitations of publication and reporting biases and short duration of follow up if more recent data from ongoing studies can be analyzed than those available in published reports. By using this method on the annual results of Mazandaran's STEPS Study, this study aims to investigate the situation of overweight and obesity status and its predisposing factors in this area.

Methods

Data sources

The data of this study was part of the STEPS Study in Mazandaran (northern Iran) which was conducted annually during 2005-2009. All information was collected using a standardized universal questionnaire(25) and contained general, demographic, Anthropometric, behavioral and clinical information of 15-64 years old people (5 age groups with 10 years interval) living in urban and rural areas of Mazandaren Province. Study sample was selected using one stage cluster sampling method in which postal areas (cities) or households (villages) considered as primary sampling units. Based on the National STEPS Project, the relevant estimated sample size in each year was 1000 except for 2005 which was 3759 subjects.

Data collection and quality assessments

Body Mass Index (BMI) was calculated through dividing the weight(Kg) by squared height (meters). Obesity was defined as BMI>30 and overweight was defined as BMI more than 25 and less than 30(21-24, 26). Ninety four teams each contained two trained interviewers collected data from study sample and 30 supervisors was selected for quality control of the study. All data entered in EPI6 software.

Statistical analysis

All data were analyzed separately and totally (by merging the 5 years data). Annual results were compared and the trends of the indicators were investigated. Descriptive statistics were conducted using mean (standard error) and percent frequency. Mean of indicators in different sexes, age groups, jobs and residency areas were compared using independent t-test and the prevalence were compared using chi square test. P value less than 0.05 was considered as significant level. In addition, a meta-analysis for estimating the pool weighted prevalence of obesity of the 5 years results was carried out considering the degree of heterogeneity of the results (Q).

All estimates were directly standardized based on real population size of different age-sex groups of Mazandaran province in national census 2006 to reduce the biases due to equal sizes of age and sex groups in the study sample. Analyses were performed using STSTA V.11.

Results

Totally 7759 subjects were recruited in this study, most of which(3759) were in 2005. The average age was 39.49, proportion of males and females and mean of age in both genders did not differ statistically significant (p=0.9).

Standardized mean weight of the total sample was 70.06 kg(males:72.86, females: 67.20, p<0.0001), mean of height was 164.25 cm (males: 170.76 and females: 157.59, p<0.0001), mean waist circumference was 87.55 cm (males: 86.37 and females: 88.76, p<0.0001) and mean of body mass index was 26.03 (males: 24.98, females: 27.11, p<0.0001). 53.7 percent (52.3-55.1) of total subjects had BMI more than 25 (overweight or

obesity), while 20.8% (19.6-22.1) of them were obese(BMI>30). Of overweight or obese subjects, 46.1% (45.1-47) were male and 61.5%(60.7-62.4) were female. Distribution of obesity between males and females was 13.5%(12.9-14.2) and 28.3%(27.4-29.1) respectively.

Prevalence of overweight status in males was 32.9%(31.8-34.1) and in females was 33.3%(32.5-34.1). Moreover, in all years of the study, the standardized prevalence of obesity in females was more than that in males (p<0.0001). The most obese patients were detected in 2007(23.2\%, 95% CI: 19.4-27.5) and the least of them were found in 2005 (19.5\%, 95% CI: 18-21.1).

The most total prevalence of overweight status in both genders was in 55-64 years age group (42.6%, 95% CI: 42.2- 42.7), while the frequency of obesity in 45-54 years old subjects of both genders was more than that of the other persons (33.5, 95% CI: 33.2-33.8). Prevalence of overweight and obesity in 15-34 year-old people in women was more than that of men in contrast to the other age groups whom prevalence of overweight in males and prevalence of obesity in females was dominant.

Except 2006, in other years, 15-24 year-old individuals had the least prevalence of obesity(7.6%; 95% CI: 6.1-9.4).(table 1)

The mean of BMI for urban residents was more than that of rural residents (25.90 vs. 26.18, p=0.07), but only in 2005 the difference was statistically significant (p=0.04). In both areas it was in the highest level in the last year of study (graph 1).

The prevalence of obesity in different jobs has demonstrated in table 2. Retireds, housewives and clerks had the most BMI mean (27.66, 27.25 and 25.60 respectively). The most prevalence of overweight and obesity was detected in student-soldiers and retireds respectively. There

Table 1. Standardized* prevalence of overweight and obesity and mean (SE) of BMI and waist circumference among age and sex groups in 2005-2009, Mazandaran STEPS Study

year								
Age group	sex		2005	2006	2007	2008	2009	Total
	Mala	overweight	16.8(15.5- 18.3)	25.8(23.5- 28.2)	17.9(15.2- 21)	28.6(25.3- 32.1)	18.2(14.5- 22.5)	19.8(18.7- 20.9)
15.24	Male	obesity	7.5 (6.6-8.5)	5.2(3.3-7.9)	14.7(12.1- 17.8)	4.1(2.9-5.7)	14.1(11.8- 16.9)	8.5 (7.8- 9.3)
13-24	famala	Overweight	26.6 (25.1- 28.2)	25.3(22.4- 28.3)	26.3(23.4- 29.4)	21.6(18.4- 25.1)	25.3(23.1- 27.5)	25.7 (24.6- 26.7)
	Ternale	obesity	9.9 (8.9- 11)	10.1(7.9-12.9)	7.4(5.7-9.4)	15.9(13.3- 18.9)	8.1(6.6-9.9)	10.1 (9.3- 10.9)
	Mala	Overweight	37.2 (35.9- 38.5)	41.8(40.3- 43.4)	36.7(34.1- 39.5)	21.6(19.2- 24.3)	33.3(31- 35.8)	35.3 (34.4- 36.1)
25.24	Male	Obesity	14 (13.1- 15)	11.2(10-12.6)	12.2(10.5- 14.2)	15.5(13.7- 17.4)	15.2(13.1- 17.4)	13.8(13.1- 14.4)
23-34	famala	Overweight	38 (36.7- 39.3)	35.6(33.4- 37.9)	37(34.6- 39.4)	45.7(43.5- 48)	30.8(28.7- 32.9)	37.6 (36.8- 38.5)
	Termale	Obesity	28.1 (27-29.3)	33.7(31-36.4)	37(34.7- 39.4)	26.7(24.5- 29)	28.8(26.5- 31.3)	29.9 (29.1- 30.7)
	Mala	Overweight	44.7 (43.8- 45.7)	36.9(35.1- 38.7)	36.6(35- 38.2)	35.7(34- 37.5)	36.4(34.7- 38)	40.5 (39.8- 41.1)
25 11	Iviale	Obesity	15.5 (14.8- 16.3)	23.3(22.1- 24.6)	24.7(23.1- 26.5)	16.3(15- 17.8)	17.2(15.9- 18.5)	18(17.5- 18.5)
55-44	Famala	Overweight	39.5 (38.6- 40.4)	38.8(36.6-41)	39(37.2- 40.9)	39.2(37.6- 40.8)	35.1(33.6- 36.5)	38.8(38.1- 39.4)
	remaie	Obesity	40.6 (39.6- 41.5)	45.9(43.2- 48.7)	40(38.2- 41.8)	42.3(40.6- 44)	49.5(48.2- 50.7)	42.5 (41.8- 43.2)
	Mala	Overweight	44.4 (43.8- 45.1)	41.5(40.3- 42.7)	41(39.8- 42.5)	46.7(45.4- 47.9)	49(47.6- 50.4)	44.4 (44- 44.9)
15 51	Wate	Obesity	16.8 (16.2- 17.3)	22.3(21.5- 23.2)	23.8(22.7- 24.9)	16.7(15.6- 17.8)	18(17.3- 18.7)	18.6 (18.2- 18.9)
43-34	Fomala	Overweight	37.6 (37- 38.2)	32(31-33)	26.8(25.6- 28)	33(31.8- 34.2)	37.8(37- 38.6)	35 (34.6- 35.4)
	remale	Obesity	45.3 (44.6- 45.9)	56(55-57)	54.6(53.2- 56.1)	4 6.4(45.2- 47.6)	$5\overline{2(51.2-52.9)}$	48.8 (48.4- 49.3)

$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$									
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			Overweight	47.3 (47-	44.9(44.4-	43.8(43.2-	46.4(45.7-	42.6(41.9-	45.8 (45.6-
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	Mala	Overweight	47.6)	45.4)	44.4)	47.1)	43.3)	46)	
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		Iviale	Obesity	15.6 (15.4-	15.3(14.8-	22.9(22.2-	17(16.5-	22.8(22-	17.6 (17.4-
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	55 61		Obesity	15.8)	15.9)	23.5)	17.5)	23.6)	17.8)
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	33-04		Overweight	40.2 (39.8-	36.4(35.6-	45.8(45.1-	38.5(37.7-	34(33.4-	39.4 (39.2-
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		fomala		40.5)	37.1)	46.5)	39.2)	34.6)	39.7)
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$		lemale	Obesity	39.9 (39.5-	12 1(11 0 13)	34.6(34-	40.4(39.7-	55(54.4-	41.5 (41.2-
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			Obesity	40.2)	42.4(41.9-45)	35.2)	41.1)	55.6)	41.7)
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			Mala	32.9 (31.7-	35.3(33.7-	30.9(28.8-	31.6(29.1-	31.1(28.5-	32.5 (31.7-
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	prev	alence	Iviale	34)	36.9)	33.2)	34.2)	33.8)	33.4)
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	of Ov	erweight	female	34.3 (33.1-	22(20, 24, 1)	32.8(30.6-	33.7(31.4-	30.7(29-	33.3 (32.5-
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $		-		35.4)	52(50-54.1)	35.1)	36)	32.4)	34.1)
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$			Total	33.6 (31.9-	22 7(21 26 5)	31.9(28.8-	32.6(29.3-	30.9(27.8-	32.9 (31.8-
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			Iotai	35.3)	35.7(31-30.3)	35.1)	36.1)	34.1)	34.1)
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			Mala	12.4 (11.5-	12(11 / 1/ 7)	17.6(15.4-	11.8(10.4-	16(14.2-	13.5 (12.9-
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $			Iviaic	13.3)	13(11.4-14.7)	20.1)	13.3)	18.1)	14.2)
obesityTenlate 27.9 $30.8(27.0-34)$ 31.4 31.6 32.1 29.1 Total 19.5 (18- $21.8(17.5 23.2(19.4 20.3(17.3 22.9(20.1 20.8$ (19.6- 21.1 26.7 27.5 23.7 25.9 22.1 Male 24.9 (0.12) $25.1(0.3)$ $25.3(0.3)$ $24.7(0.2)$ $25.2(0.2)$ 24.98 (0.09)female 26.9 (0.14) $27.4(0.4)$ $27.01(0.3)$ $27.1(0.3)$ $27.4(0.3)$ 27.11 (0.1)Total 25.90 $26.24(0.31)$ $26.16(0.29)$ 25.89 (0.21) 26.29 26.03 (0.16)(0.08)	preval	lence of	famala	26.8 (25.8-	20 8(27 8 24)	28.9(26.6-	29.1(26.7-	29.9(27.7-	28.3 (27.4-
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	ob	esity	Iemaie	27.9)	30.8(27.8-34)	31.4)	31.6)	32.1)	29.1)
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$			Total	19.5 (18-	21.8(17.5-	23.2(19.4-	20.3(17.3-	22.9(20.1-	20.8 (19.6-
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$			Total	21.1)	26.7)	27.5)	23.7)	25.9)	22.1)
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$			Mala	24.0(0.12)	25.1(0.2)	25.2(0.2)	24.7(0.2)	25 2(0 2)	24.98
$\begin{array}{ c c c c c c c c c c c c c c c c c c c$			Iviale	24.9 (0.12)	23.1(0.3)	23.3(0.3)	24.7(0.2)	23.2(0.2)	(0.09)
Total 25.90 (0.11) $26.24(0.31)$ $26.16(0.29)$ $25.89(0.21)$ 26.29 (0.16) 26.03 (0.08)	BMI m	nean (SE)	female	26.9 (0.14)	27.4(0.4)	27.01(0.3)	27.1(0.3)	27.4(0.3)	27.11 (0.1)
(0.11) 20.24(0.31) 20.10(0.27) 23.07(0.21) (0.16) (0.08)		. ,	Total	25.90	26.24(0.31)	26 16(0 20)	25.80 (0.21)	26.29	26.03
			10101	(0.11)	20.24(0.31)	20.10(0.29)	23.09 (0.21)	(0.16)	(0.08)

*Based on Mazandaran national census 2006

Table 2. Standardized* Prevalence and 95% confidence interval of obesity in different job groups in 2005 - 2009

year	2005	2006	2007	2008	2009	Total
Job						
Clerk	16(13.1-	20.9(13.6-	12.7(8.7-	15.7(10.4-	14.6(9.8-	16.5(14.1-
CICIK	19.4)	30.7)	18.4)	23)	21.2)	19.1)
	16.7(9.9-	9.1(3.7-	7(5,0,7)	12.2(7.5-	2.9(2.4.(.1))	15.4(10.4-
Student/ soldier	26.7)	20.9)	/(5-9.7)	19.1)	3.8(2.4-0.1)	22.1)
I Janaa madu	28.4(22.1-	34.1(30.1-	32(29.5-	33.4(24.5-	41.5(39.4-	31.2(22.1-
House work	35.7)	38.2)	34.7)	43.7)	43.7)	42)
Datinad	28(25.1-	$(0) \Lambda(((\Lambda)))$	46.3(42.9-	65.9(62.9-	18.1(16.6-	34.4(31.5-
Keurea	31.2)	09.4(00.4)	49.7)	68.9)	19.7)	37.4)
o th or	10.6(6.1-	8.8(5.3-	12.8(6.6-	9.7(5.2-	22.9(12.7-	12.8(9.3-
other	17.7)	14.2)	23.4)	17.4)	37.6)	17.3)
P- value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

*Based on Mazandaran national census 2006

were statistically significant difference in BMI mean among genders, age groups, jobs and area of residency (p<0.0001). During these years, the mean (standard error) of BMI has increased from 25.9(0.21) in 2005 to 26.3(0.16) in the last year.

In this individual meta-analysis of the 5 years results, using fixed model with no heterogeneity of results (Q=6.53, p=0.2), the pooled prevalence of obesity was estimated about 20.76(95% CI: 19.63-21.96) (graph 2), also the pooled Mean(SE) of BMI and waist circumstan-

ce estimated about 26.03(0.08) and 87.55(0.2) respectively.

Discussion

Our study showed that prevalence of Obesity and overweight status in Mazandaran Province is considerably higher than that of in most of the other studies and also Obesity was more in women, urban residents, Housewives and retireds. This study also showed that Prevalence of obe-



Graph 1. Mean of BMI in urban and rural residence in 2005-2009

sity and overweight status increased by age and only prevalence of obesity in women has had an growing trend over time.

Prevalence of obesity and overweight in this study was about 20.8% and 32.9% respectively, which was slightly more than the rates reported by Hajian(21), Talaee(27) and the average prevalence of Iran(22). It also was very higher than that of Rafsanjan(southern Iran)(24) and Seri Lanka(28). Only the prevalence estimated in Golestan (another northern province of Iran) was more than that of found in the current study(18). The results for BMI in this study also was similar to the results of Iran(22) but was more than that of Serilanka(28). The big difference between the results of northern and southern parts of Iran might be due to different culture and life styles such as more calorie intake and low physical activity in northern residents.

The prevalence of obesity in the current study in females were significantly more than that of males. This finding was similar to the most other studies (22,23,24, 27, 28). For example in a study carried out in Khouzestan(south-west Iran), the odds of overweight status and obesity in women was 2.6 and 5.6 fold greater than that of males(23). Some reason for this difference could be early marriage, high level of stress, low physical activity, sextual hormones, nutritional behavioral imbalances and frequent pregnancies(23).

The results of this IPD Meta-Analysis study showed that prevalence of overweight and obesity status had an increasing trend by age. This was in keeping with the results of a study in southern of Iran which showed that the most prevalence of obesity was in more than 51 year-old women and



Graph 2. Forest plot for prevalence of obesity (Mazandaran steps study 2005-2009)

31-50 year-old men(24) and also findings of Hajian (21) and Mirza zadeh(24) detecting 40-59 yearold subjects as the most obese people. Similarly, a national survey in Pakistan showed that mean of BMI increased by aging and prevalence of obesity in 45-64 age group was two folds more than that of 18-35 years age group(1).

In our study, The mean of BMI and prevalence of obesity and overweight status in urban residents was more than rural residents although that was not statistically significant. These results are similar to the results of other studies conducted in northern Iran(18) and Pakistan(1).

But the important problem is the statistically significant growing trend of BMI Mean in rural areas during these 5 years. It might be due to remarkable changes in the life style of rural people and development of urbanization within rural areas leading to increasing socioeconomic problems in the community

The results of the current study showed that the most prevalence of obesity was in retireds and housewifes. May be lower physical activity in these subjects is the main cause. Hajian et al found that risk of obesity in persons with severe occupational activity was 56% less than the other job groups who had low levels of activity(21).

In conclusion, This individual meta- analysis showed that a high portion of people living in northern of Iran suffer from obesity and overweight status. It seems that the most obese people of Iran are living in its northern provinces. The least prevalence of physical activity and fruit-vegetable consumption in this area which has been shown in another study(22) could be an explanation for that result. Different studies in the world has found a growing trend of obesity in the recent decades(29-31). Similar to the current study, another research in capital of Iran showed an increasing trend of obesity and overweight which was statistically significant during a 5 years period(24). Such an epidemic might be due to urbanization, changing in life style and economic changes, development of scientific and machinery agriculture and lower physical activity during agricultural tasks and more calorie intake because of higher race consumption. These results indicate a rapid but silent growth of obesity. Therefore it needs that educational and research centres and health providers make suitable policies and strategies in order to prevent this problem. For instance, interventional programs for modification of nutritional behaviours should be taken into account.

Reference

- 1. Shah SM, Nanan D, Rahbar MH, Rahim M, Nowshad G. Assessing obesity and overweight in a high mountain Pakistani population. Trop Med Int Health, 2004; 9: 526-32.
- Yologlu S, Sezgin AT, Ozdemir R, Sezgin N, Colak C, Topal E, et al. Identifying risk factors in a mostly overweight patient population with coronary artery disease. Angiology, 2003; 54: 181-6.
- 3. Kathryn B, Louis L. Obesity and the metabolic syndrome. American Journal of Critical Care, 2003;12:167-70.
- 4. Rao SV, Donahua M, pi-Sunyer FX. Results of Expert Meetings: Obesity and Cardiovascular Disease: Obesity as a risk factor in coronary artery disease, Am Heart J, 2001; 142:1102-7.
- 5. Seidell JC. The impact of obesity on health status: some implications for health care costs. Int J ObesRelatMetabDisord, 1998; 19(6):13–16.
- 6. Groessl EJ, Kaplan RM, Barrett-Connor E, Ganiats TG. Body mass index and quality of well- being in a community of older adults. Am J Prev Med, 2004;26: 126–129.
- 7. Urek R, Crncevic-Urek M, Cubrilo-Turek M. Obesitya global public health problem. Acta Med Croatica, 2007;61:161-64.
- Wolf AM, Colditz GA. Current estimates of the economic cost of obesity in the United States. Obes Res, 1998; 6: 9701–9706.

- 9. Eckel RH, Krauss RM. American heart association call to action: obesity as a major risk factor for coronary heart disease. Circulation, 1998; 97: 2099– 2100.
- Jafar TH, Chaturvedi N, Pappas G. Prevalence of overweight and obesity and their association with hypertension and diabetes mellitus in an Indo-Asian population. Canadian Medical Association Journal. 2006;175(9):1071.
- 11. Kunz I, Schorr U, Klaus S, Sharma AM. Resting metabolic rate and substrate use in obesity hypertension, Hypertension, 2000;36: 26-32.
- 12. Gharipour M, Mohamadifard N, Asgary S,Naderi GH .The prevalence of obesity and cardiovascular risk factor in Isfahan. The Journal of Qazvin University of Medical Sciences 2003;26:53-64.
- 13. Fontaine KR, Redden DT, Wang C, Westfall AO and Allison DB. Years of Life Lost Due to Obesity. JAMA, 2003;289: 187-93.
- 14. Sturm R. Increases in clinically severe obesity in the United States, 1986-2000. Arch Intern Med, 2003; 163: 2146-8
- 15. King T, Kavanagh AM, Jolley D, Turrell G and Crawford D. Weight and place: a multilevel crosssectional survey of area-level social disadvantage and overweight/obesity in Australia. International journal of obesity. 2005;30(2):281-7.
- 16. James PT. Obesity: the worldwide epidemic. Clinics in Dermatology, 2004; 22(4): 276-280.
- 17. Bessesen DH. Update on obesity. J Clin Endocrinol Metab, 2008;93(6):2027-4.[PMID=18539769]
- 18. Abdollahi AA, Behnampour N, Vaghari G, Bazrafshan H. The Correlation Between Age, Gender and Education with Obesity in Urban Population of Golestan Province, Iranian Journal of Endocrinology and Metabolism,2011;12(3): 276-282.
- 19. Khatib O. Non communicable diseases: risk factors and regional strategies for prevention and care, East Mediterr Health J, 2004;10(6):778-88. [PMID=16335764]
- 20. Balkau B, Deanfield JE, Despres JP, Bassand JP, Fox KA, Smith SC, et al. International Day for the Evaluation of Abdominal Obesity (IDEA): a study of waist circumference, cardiovascular disease, and diabetes mellitus in 168,000 primary care patients in 63 countries. Circulation, 2007;116: 1942-51.

- 21. HajianTilaki KO, Heidari B. Prevalence of obesity, central obesity and the associated factors in urban population aged 20–70 years, in the north of Iran: a population based study and regression approach. Obesity reviews. 2007;8(1):3-10.
- 22. Mirzazadeh A, Haghazali M, Esghari F and Aghajani H. Non-Comunicable Diseases Risk Factors Surveillance Provincial Report Islamic Republic Of Iran-2007, publication: the Center of Disease Control & Management, Ministry of Health and Medical Education Deputy for Health, 2010.
- 23. Naghashpour M, Shakerinejad GH, Haghighizadeh MH, Hajinajaf S, Jarvandi F. Prevalence of Obesity and its Association with Demographic Indices in Referents to University Jahad Khozestan Clinic, Scientific Medical Journal, 2011;10(1).
- 24. Mirzazadeh A, Sadeghirad B, Haghdoost AA, Bahreini F, Kermani MR. The prevalence of obesity in Iran in recent decade; a systematic review and meta-analysis study. Iranian Journal of Public Health. 2009;38(3).
- 25. Bonita R, de Courten M, Dwyer T, Jamrozik K, Winkelmann R. Surveillance of risk factors for noncommunicable diseases: The WHO STEPwise approach. Summary.Geneva: World Health Organization, 2001.
- 26. Khaodhiar L, Blackburn GL. Obesity assessment. Am Heart J, 2001; 142: 1095–1101.
- 27. Talaei A, Amini M, Alikhani S, Delavari A, Mahdavi A. Waist circumference Cut off in Relation to Hypertension in Iran. Iranian Journal of Endocrinology and Metabolism, 2008; 4: 375-82.
- Wijewardene K, Mohideen MR, Mendis S, Fernando DS, Kulathilaka T and Uluwitta P. Prevalence of hypertension, diabetes and obesity: baseline findings of a population based survey in four provinces in Sri Lanka, Ceylon Medical Journal, 2010;50(2): 62-70.
- 29. Khambalia AZ, Seen LS.Trends in overweight and obese adults in Malaysia (1996–2009): a systematic review, Obesity review, 2010;11(6):403-412.
- 30. Xi B, Liang Y, He T, Reilly KH, Hu Y, Wang Q, et al.Secular trends in the prevalence of general and abdominal obesity among Chinese adults, 1993– 2009, Obesity review, 2012;13(3):287-296.
- 31. Rokholm B, Baker JL, Sorensen TIA. The levelling off of the obesity epidemic since the year 1999 – a review of evidence and perspectives, Obesity review, 2010;11(12):835-846.

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Prevalence of risk factors for skin cancer in civil construction workers in Brazil

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Abstract

Skin cancers are the malignant neoplasms with the highest estimated prevalence rates in the world. Skin cancer risk factors include sun exposure, light complexion, occupation, family history of skin cancer, smoking habits and no use of sunscreen or personal protective equipment. Civil construction workers are subjected to sun exposure for long periods of time. This quantitative observational study aimed at determining the prevalence of skin cancer risk factors in civil construction workers in Brazil. Interviews were carried out with 72 workers involved in the construction of a hospital in Northeastern Brazil, a semi-arid environment. The majority of the sample had brown or black skin, a protective factor in itself. However, 72.2% of the interviewed workers did not use sunscreen, and the majority did not wear personal protective equipment. Smoking habits were reported by 31.9% of them, and 51% of the non-smoking individuals used to smoke in the past. Long-term sun exposure, non-use of sunscreen and smoking habits were the main risk factors found in the studied population. It is necessary to improve the understanding of both civil construction employers and employees about the importance of using personal protective equipment, so as to reduce skin cancer risks from occupational sun exposure.

Keywords: Skin neoplasms, Melanoma, radiation effects.

Introduction

Skin cancers are the malignant neoplasms with the highest estimated prevalence rate in the world. Skin cancers are either non-melanoma or melanoma. Non-melanoma skin cancers occur in either squamous cells or in basal cells, the latter being the most common type. In Brazil, National Cancer Institute (INCA) estimates that 62.280 men and 71.490 women will be diagnosed with non-melanoma skin cancer in 2012¹.

When compared to basal cells carcinomas, despite being less frequent, squamous cells carcinomas have higher mortality rates, as well as higher risks of metastasis². Melanoma malignant skin cancer has become a public health issue in many countries, especially those in which the majority of the population has fair skin. In Europe, since 1960, the incidence of melanoma skin cancer has been increasing 3% to 8% per year, showing higher rates among the elderly³. The World Health Organization estimates that, annually, there are between 2 and 3 million new cases of cancer worldwide. Melanoma cases are responsible for 132.000 cases, indicating a prevalence of 2.5%⁴.

Sun's ultraviolet-B radiation (UV-B) is a risk factor for the 3 types of skin cancer herein studied. This is especially evident when analyzing the risk of having melanoma⁵. Some outdoor workers, e.g. postmen, civil construction workers and farmers, are highly affected by the high levels of UV-B radiation received⁶, that is worsened by the lack of protection and instruction. These workers are, then, constantly exposed to the main skin cancer risk factor in population samples, being an important social problem with relevant consequences to public health, considering that skin cancer is the most prevalent in Brazil¹. Despite being a controversial matter, in order to understand the risk of developing a skin cancer, either melanoma or non-melanoma, it is important to take into account the person's complexion, use of sunscreen, family history of skin cancer and life habits (as smoking habits). The purpose of this study was to study the prevalence of skin cancer risk factors in a population of civil construction workers in Brazil.

Methods

A cross-sectional quantitative survey was conducted in a population of civil construction workers involved in the construction of a hospital in the city of Juazeiro do Norte, in Northeastern Brazil, a tropical semi-arid environment.

Data was gathered using a questionnaire, which analyzed the presence or not of skin cancer risk factors in the studied population. Information obtained during the interview included: age; skin color (classified in yellow, white, black and brown/mixed, according to the 2009 classification of the Brazilian Institute of Geography and Statistics, IBGE); eyes and hair; years of work at the in the company; work schedule; occupational history; use of sunscreen and individual protective equipment; family history of cancer and smoking habits. The questionnaire used in the survey was gently provided by PhD Regina Célia Popim, nurse from the Nursing Department of the Medical School of Paulista State University Julio de Mesquita Filho (UNESP)⁷. The questionnaire was slightly modified, in order to fit the purposes of the research. The data collected were organized in Microsoft Excel and statistically analized with the software SPSS®, version 17.0.

Results and discussion

A total of 72 civil construction workers were interviewed, all of them male. The studied population had an occupational sun exposure of 8h/day, which represents a relevant risk factor for the 3 types of skin cancers (melanoma⁸, basal cells carcinoma and squamous cells carcinoma). Occupational exposure to radiation is an important risk factor for skin cancer and other skin illnesses, because it gets workers in contact with UV radiation for long periods of time, sometimes without the necessary protective equipment. Regarding skin color, 2.8% had yellow skin; 16.7%, white skin; 31,9% had black skin e 48.6%, brown/mixed skin. It is important to highlight that workers with white skin (16.7%) have a higher risk for skin cancer, either basal cells carcinoma, squamous cells carcinoma or melanoma¹⁰. 89.9% of the workers had brown eyes, while 4.2% had blue eyes and 6.9% had green eyes. For hair color, the study found the

Skin color	Frequency	Relative Frequency (%)	Cumulative Frequency (%)
Yellow	2	2,8%	2,8%
White	12	16,7%	19,4%
Black	23	31,9%	51,4%
Brown/mixed	35	48,6%	100,0%
Total	72	100,0%	

Table 1. Frequency distribution of skin color in civil construction workers in Brazil

Table 2.	Frequency	distribution	of eye	color in	civil	construction	workers	in Brazil
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Eye Color	Frequency	Relative Frequency (%)	Cumulative Frequency (%)
Blue	3	4,2%	4,2%
Brown	64	88,9%	93,1%
Green	5	6,9%	100,0%
Total	72	100,0%	

Table 3.	Frequency	distribution	of	hair	color	of	civil	construction	workers	s in	Bro	azil
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Hair color	Frequency	Relative Frequency (%)	Cumulative Frequency (%)
Brown	31	43,1%	43,1%
Blond	1	1,4%	44,5%
Black	40	55,5%	100,0%
Total	72	100,0%	

Equipment	Frequency	Relative Frequency (%)	Cumulative Frequency (%)
None	34	47,2%	47,2%
Сар	7	9,7%	56,9%
Long trousers	6	8,3%	65,2%
Hat	1	1,4%	66,6%
More than one	19	26,4%	93,0%
Long-sleeved shirt	1	1,4%	94,4%
Others	4	5,6%	100,0%
Total	72	100,0%	

Table 4. Individual protective equipments used by civil construction workers in Brazil

Table 5. Frequency distribution of sunscreen use by civil construction workers in Brazil

Sunscreen Use	Frequency	Relative Frequency (%)	Cumulative Frequency (%)
No	52	72,2%	72,2%
Yes	20	27,8%	100,0%
Total	72	100,0%	

Table 6. Frequency distribution of sunscreen use and non-use causes by civil construction workers in Brazil

Sunscreen use and reasons for non-use	Frequency	Relative Frequency (%)	Cumulative Frequency (%)
Use	20	27,8%	27,8%
Do not use (high costs of sunscreen)	5	6,9%	34,7%
Do not use (lack of habit)	35	48,6%	83,3%
Do not use (did not receive proper orientation)	9	12,5%	95,8%
Do not use (other reasons)	3	4,2%	100,0%
Total	72	100,0%	

Table 7. Frequency distribution of cigarrette smoking habit of civil construction workers in Brazil

Cigarrette Smoking Habit	Frequency	Relative Frequency (%)	Cumulative Frequency (%)
No	49	68,1	68,1
Yes	23	31,9	100,0
Total	72	100,0	

following frequencies: 43.1 had brown hair, 1.4% had blond hair and 55.5% had black hair (Tables 1, 2 and 3). The average age of the sample was 37.79 years-old, with a mode of 31 years-old. The youngest worker interviewed was 21 years-old, whereas the eldest had 71 years-old. Results concerning years of work at the in the company showed that 91.7% of the sample worked for the current civil construction company for less than 6 months. civil construction worker (23.6%), ironworker (12.5%), servant (11.1%) and carpenter (23.6%). All other previous occupations were related to prolonged sun exposure, like doing outdoor services or working as a farmer. Regarding work schedule on the current job, 87.5% worked 8 hours per day, 6 days per week (from Monday to Saturday). The others (12.5%) worked 8 hours per day, 7 days per week (from Monday to Sunday). About the use of individual protective equipments, it was

The most common previous occupations were

found that 74.2% did not wear individual protective equipments, whereas 52.8% wore some kind of protection, most of them of doubtful efficacy. From these, 9.7% wore cap; 8.3% wore long trousers; 1.4% wore hat; 1.4% wore long-sleeved shirt; 5.5% wore other type of equipment e 26.4% wore more than one type of equipment (Table 4).

Regarding sunscreen use, 72.2% of the total sample did not use sunscreen (Table 5). Among the 27.8% who used sunscreen, 65% used it at a daily basis, whereas 35% used it rarely. In the population who did not use sunscreen, 67.3% claimed not using it out of lack of habit; 9.6% reported the high costs to buy sunscreen as an obstacle to buying the product (Table 6). Among the workers who used sunscreen, 55% knew the Solar Protection Factor (SPF) of the product, 35% of the products used being of SPF 30.

44.4% of the interviewed workers reported doing skin self-examination on a regular basis, whereas 55.6% declared not to do it.

About family history of cancer, 4.2% of the workers reported at least one cancer case in the family. 87.5% denied having a case of cancer in the family.

Cigarette smoking, a habit socially widespread, enhances mainly lung and oral cancer risks, and, in a shorter scale, skin cancer^{11,12}. Cigarettes bear various carcinogenic agents, such as nicotine, lead, benzene and arsenic¹³. When asked about cigarette smoking habits, 68.1% reported being nonsmokers, and 31.9% declared themselves smokers (Table 7). 27.2% of the latter were smokers for 15 to 18 years, and 30.4% smoked 20 cigarettes a day. In addition, 65.2% only smoked filtered cigarettes, 17.4% smoked only non-filtered cigarettes and 17.4% smoked cigarettes of both types. 51% of the non-smoking individuals used to smoke in the past.

Conclusion

In this study, daily sun exposure of the sample due to occupational reasons is an important risk factor for skin cancers. Another relevant risk factors are the absence of regular sunscreen use and cigarette smoking habits.

Regarding subjects' complexion, the majority of the population had black or brown/mixed skin,

what owned them a certain level of natural protection. Frequency distribution of non-susceptible individuals regarding the person's complexion appeared, then, on higher levels.

The civil construction working company for which the individuals of the sample worked did not distribute the adequate individual protective equipment, designed to protect workers in case of prolonged sun exposure. It is necessary to improve the understanding of both civil construction employers and employees about the importance of using personal protective equipment, as well as the government surveillance of the correct use and distribution of these equipments, so as to reduce skin cancer risks caused by occupational sun exposure.

References

- 1. Instituto Nacional do Câncer (Brasil). Estimativa 2012: incidência de câncer no Brasil. Rio de Janeiro (RJ): INCA; 2011.
- 2. Ramos J, Villa J, Ruiz A, Armstrong R, Matta J. UV dose determines key characteristics of nonmelanoma skin cancer. Cancer Epidemiol Biomarkers Prev 2004; 2004;13(12).
- 3. Thompson JF, Scolyer RA, Kefford RF. Cutaneous melanoma. Lancet 2005; 365: 687-701.
- 4. World Health Organization. Ultraviolet radiation and the INTERSUN Programme [internet] .Genebra: The Organization; 2012 . [accessed 2012 May 07]. Disponível em: http://www.who.int/uv/faq/skincancer/en/ index1.html.
- 5. Markovic SN, Erickson LA, Rao RD. et al. Malignant melanoma in the 21st Century, part 1: epidemiology, risk factors, screening, prevention, and diagnosis. Mayo Clin Proc 2007;82 (3) 364-80.
- Szklo AS, Almeida LM, Figueiredo V, Lozana JA, Mendonça GAS, Moura L, et al. Comportamento relativo à exposição e proteção solar na população de 15 anos ou mais de 15 capitais brasileiras e Distrito Federal, 2002-2003. Cad. Saúde Pública 2007;23:823-34.
- 7. Popim RC, Corrente JE, Marino JAG, Souza CA. Câncer de pele: uso de medidas preventivas e perfil demográfico de um grupo de risco na cidade de Botucatu. Ciênc Saúde Coletiva 2008;13(4):1331-6.

- Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P, Melchi CF. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer 2005 Jan;41(1):45-60.
- 9. Glanz K, Buller DB, Saraiya M. Reducing ultraviolet radiation exposure among outdoor workers: state of the evidence and recommendations. Environ Health 2007 Aug; 8(6): 22.
- 10. Zanetti R, Rosso S, Martinez C, Nieto A, Miranda A, Mercier M, et al. Comparison of risk patterns in carcinoma and melanoma of the skin in men: a multi-centre case-case-control study. Br J Cancer 2006 Mar; 94(5):743-51.
- Sartor, SG. Occupational risks for laryngeal cancer: a case-control study. Cad. Saúde Pública 2007 Jun; 23(6): 1473-81.
- 12. Menezes AMB, Horta BL, Oliveira ALB, Faufmann RAC, Duquia R, Diniz A et al. Risco de câncer de pulmão, laringe e esôfago atribuível ao fumo. Rev de Saúde Pública 2002; 36(2): 129-34.
- 13. Cunha GH, Jorge ARC, Fonteles MMF, Sousa FCF, Viana GSB, Vasconcelos SMM. Nicotina e tabagismo. Rev Elet Pes Méd 2007 Nov; 1(4): 1-10.
- Ferrari Júnior NM, Muller H, Ribeiro M, Maia M, Sanchez Júnior JA. Cutaneous melanoma: descriptive epidemiological study. Med J 2008 Jan; 126(1): 41-7.

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Relationship of basal metabolic rate with age, body mass index, waist circumference, fat mass, and fatfree mass in African American college students

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Abstract

The purpose of this study was to examine the relationship between basal metabolic rate (BMR), age, body mass index (BMI), waist circumference (WC), fat mass (FM), and fat-free mass (FFM) in African American college students. The study included 167 African American college students aged 17-39 years who visited the participating exercise physiology laboratory in North Carolina at the Agricultural and Technical State University, Greensboro, NC, United States, during 2011. BMR was measured using a BMR analyzer (Inbody 720, Seoul, Korea), and the age, BMI, and WC data from each subject were recorded and incorporated into subsequent analyses. Body composition measures such as FM and FFM were determined using air-displacement plethysmography (BOD POD, Life Measurement Inc., California, USA). Subsequently, the relationship between BMR and the other variables examined in this study (age, BMI, WC, FM, and FFM) were assessed using Pearson's r correlation analysis. BMR was not significantly correlated with age (p > 0.05) but was positively correlated with BMI (male: r = 0.664, p < 0.001; female: r = 0.475, p < 0.001), WC (male: r = 0.669, p < 0.001; female: r = 0.481, p < 0.001), FM (male: r = 0.642, p < 0.001; female: r = 0.455, p < 0.001), and FFM (male: r = 0.879, p < 0.001; female: r = 0.861, p < 0.001). We concluded that while BMI, FFM, FM, and WC were all significantly correlated with BMR in African American students (both male and female), the correlation between BMR and FFM was the highest. Furthermore, whereas BMR and FFM were correlated in males and females to a similar degree, the correlation of BMR with BMI, WC, and FM was higher in male than in female students.

Keywords: Basal metabolic rate, Body mass index, Fat free mass, Fat mass, Waist circumference, African American.

Introduction

According to the Center for Disease Control and Prevention (CDC), in 2011, approximately 33.8% of adults and approximately 17% of children and adolescents aged 2–19 years in the United States of America were obese; moreover, the incidence of obesity is continually increasing every year (1). Obesity is mainly caused by a mismatch between energy expenditure and energy intake (2-3). The factors related to this balance are complex and numerous and include the socioeconomic environment, genes, family history, sedentary lifestyle, eating habits, and the interaction between these factors (4).

The most effective weight-control strategy is to identify and modify the relative amounts of energy expenditure and energy intake (5). Particularly, energy expenditure is a determining factor of traits related to body composition, such as obesity and energy balance. Daily energy expenditure (DEE) is accounted for by basal metabolic rate (BMR), which accounts for approximately 65% of DEE, physical activity (or the thermic effect of activity), which accounts for approximately 25%, and the thermic effect of food (including digestion, absorption, and metabolism of nutrients), which accounts for approximately 10% (6-7). Because BMR is the largest contributor to DEE, it has been the focus of research on the treatment and prevention of obesity.

Generally, BMR is mainly dependent on aspects of body composition such as fat-free mass (FFM) and fat mass (FM), body mass index (BMI), age, and waist circumference (WC) (8-12). Most previous studies have mainly been performed on Caucasians (13-14), and data on the relationship between BMR and associated factors in other races such as African Americans are lacking. Therefore, the purpose of this study was to examine the relationship of BMR with age, BMI, WC, FM, and FFM in African American college students.

Methods

Subjects

This study included 167 African American college students aged 17–39 years who visited the participating exercise physiology laboratory in North Carolina Agricultural and Technical (A&T) State University in Greensboro, NC, United States, between June 1, 2011 and December 31, 2011. The BMR, age, BMI, WC, FM, and FFM of all subjects were determined. All study procedures were approved by the institutional review board at North Carolina A&T State University. The characteristics of the subjects are shown in Table 1.

Experimental procedures

The BMR of each subject was measured using a BMR analyzer (Inbody720, Seoul, Korea). Age and gender were self-reported via a questionnaire. The BMI (kg/m2) of the subjects was calculated on the basis of their weight and height. The WC of each subject was measured in the trunk region midway between the lower costal margin (bottom of the lower rib) and the iliac crest (top of the pelvic bone), with the subject standing with their feet spread approximately 25–30 cm apart. The examiner stood beside the subject and measured the WC by carefully fitting the tape around the subject's trunk without compressing any underlying soft tissues. WC was measured at the end of a normal expiration to the nearest 0.5 cm (15).

Aspects of body composition such as FM and FFM were evaluated using BOD POD version 1.69 (Life Measurement Inc., Concord, California, USA). Chamber-pressure amplitudes were calibrated before each test using a 50-L calibration cylinder. Subjects were asked to wear a tightfitting swimsuit or body suit, and the percentage of body fat (%BF) was determined in the chamber. To measure thoracic gas volume, the subject was asked to sit quietly in the BOD POD chamber and breathe through a disposable tube and filter connected to the reference chamber at the rear of the BOD POD apparatus. The airway was occluded midway during exhalation after 4 or 5 breaths, and the subject was asked to blow 3 quick, light, panting breaths into the tube.

Statistical analysis

All results from this study are represented as mean \pm standard deviation. Pearson's r correlation was calculated to examine the relationship between BMR and other variables such as age, BMI, WC, FM and FFM. Statistical significance was set at p < 0.05, and all analyses were performed using SPSS version 18.0 (SPSS, Chicago, IL, USA).

Results

The correlation of BMR with age, BMI, WC, FM, and FFM is shown in Table 2. For the "all subjects" dataset, BMR was not significantly correlated with age (p > 0.05). However, BMR showed significant positive correlations with BMI (male: r = 0.664, p < 0.001; female: r = 0.475, p < 0.001), WC (male: r = 0.669, p < 0.001; female: r = 0.481, p < 0.001), FM (male: r = 0.642, p < 0.001; female: r = 0.455, p < 0.001), and FFM (male: r = 0.879, p < 0.001; female: r = 0.861, p < 0.001).

Discussion

This study evaluated the relationship between BMR and other variables such as age, BMI, WC, FM, and FFM in African American students. Previous studies have reported a reduction in BMR in elderly individuals as compared to that in younger subjects and that this age-related decline in BMR is due to a reduction in FFM (16-17). However, in the current study, because all the subjects were relatively young college students, no relationship between BMR and age was evident. Additional well-designed studies incorporating wide age ranges are required to examine the relationship between age and BMR in African American people. Lazzer et al. (2010) have reported that prediction equations based on anthropometric parameters such as WC and BMI, which is based on height and weight, and those based on aspects

Variable	Male students (n = 72)	Female students (n = 95)
Age, years	21.64 ± 2.87	19.99 ± 3.02
Height, cm	176.72 ± 6.67	165.96 ± 8.71
Weight, kg	81.82± 14.47	69.20 ± 13.97
BMI, kg/m ²	26.21 ± 4.31	25.04 ± 4.09
FFM, kg	65.74 ± 7.49	51.19 ± 7.75
FM, kg	16.08 ± 9.45	18.03 ± 9.66
WC, cm	83.75 ± 9.56	80.95 ± 9.70
BMR, Kcal	1846.19 ± 186.79	1480.03 ± 192.95

Table 1. Characteristics of the subjects (n = 167)

BMI, body mass index; FFM, fat-free mass; FM, fat mass; WC, waist circumference; BMR, basal metabolic rate

Table 2. Correlation of BMR with age, BM	, WC, FM, and FFM in Africa	can American college stud	lents (n = 167)
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Catagory	BMR in mal	e students (n = 72)	BMR in female students (n = 95)		
Category	r	р	r	р	
Age, years	0.196	0.196	-0.038	0.714	
BMI, kg/m ²	0.664	< 0.001***	0.475	< 0.001***	
WC, cm	0.669	< 0.001***	0.481	< 0.001***	
FM, kg	0.642	< 0.001***	0.455	< 0.001***	
FFM, kg	0.879	<0.001***	0.861	< 0.001***	

***p < 0.001 by Pearson's r correlation analysis

BMR, basal metabolic rate; BMI, body mass index; WC, waist circumference; FM, fat mass; FFM, fat-free mass

of body composition such as FM and FFM had the same R2 and similar root mean squared error of the estimate values (13). This implies that not only FM and FFM but also BMI and WC are positively correlated with BMR. Our results, which showed a correlation of BMR with anthropometric variables (BMI and WC) and body composition aspects (FM and FFM) in African American students, are in accordance with those of previous studies on Caucasian subjects. Interestingly, our results showed that although BMR and FFM exhibit similar relationships in both male and female students, the correlation of BMR with BMI, WC, FM, and FFM was higher in male than in female students (Table 2). Thus, gender is a significant determinant of BMR in African American college students. This study has limitations. Because the study only included subjects from North Carolina at Greensboro, it may not be representative of the wider African American college student population in the USA. Moreover, the number of subjects that participated in this study (N = 167) does not constitute a large sample size. However, we believe that the value of this study lies in the fact that it was conducted exclusively on subjects of African-American descent.

Conclusion

We conclude that BMI, FFM, FM, and WC were highly correlated with BMR in African American students (both male and female) and that the correlation between BMR and FFM was the highest. Furthermore, whereas BMR and FFM are similarly correlated in both genders, the correlation of BMR with BMI, WC, and FM was higher in male than in female students. Thus, gender is a significant determinant of BMR in African American people.

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References

- 1. Centers for Disease Control and Prevention. U.S. Obesity Trends. Centers for Disease Control and Prevention. 2011. http://www.cdc.gov/obesity/data/trends.HTML
- 2. Bouchard C, Blair SN. Introductory comments for the consensus on physical activity and obesity. Med Sci Sports Exerc. 31 (11 Suppl): S498–S501. 1999.

- 3. Flatt JP. Macronutrient composition and food selection. Obes Res. 9 (Suppl 4): S256–S262. 2001.
- 4. Thomas AW, Albert JS. Handbook of obesity treatment (3rd ed.). New York: Guilford Press. USA, 2002.
- World Health Organization. Obesity and Overweight. Global Strategy on Diet, Physical Activity and Health. 2011. http://www.who.int/mediacentre/factsheets/ fs311/en/
- 6. Foster GD, McGuckin BG. Estimating resting energy expenditure in obesity. Obes Res. 9 (Suppl 5): S367– S372. 2001.
- 7. Goran MI. Energy metabolism and obesity. Med Clin North Am. 84: 347–362. 2000.
- Wang Z, Heshka S, Wang J, Gallagher D, Deurenberg P, Chen Z, Heymsfield SB. Metabolically active portion of fat-free mass: a cellular body composition level modeling analysis. Am J Physiol Endocrinol Metab. 292: E49–E53. 2007.
- 9. Johnstone AM, Murison SD, Duncan JS, Rance KA, Speakman JR. Factors influencing variation in basal metabolic rate include fat-free mass, fat mass, age, and circulating thyroxine but not sex, circulating leptin, or triiodothyronine. Am J Clin Nutr. 82: 941–948. 2005.
- Goran MI, Kaskoun M, Johnson R. Determinants of resting energy expenditure in young children. J Pediatr. 125: 362–367. 1994.
- 11. Fukagawa NK, Bandini LG, Young JB. Effect of age on body composition and resting metabolic rate. Am J Physiol. 259: E233–E238. 1990.
- 12. Keys A, Taylor HL, Grande F. Basal metabolism and age of adult man. Metab Clin Exp. 22: 579–587. 1973.
- 13. Lazzer S, Bedogni G, Lafortuna CL, Marazzi N, Busti C, Galli R, De Col A, Agosti F, Sartorio A. Relationship between basal metabolic rate, gender, age, and body composition in 8,780 white obese subjects. Obesity (Silver Spring). 18 (1): 71-78. 2010.
- Weyer C, Snitker S, Rising R, Bogardus C, Ravussin E. Determinants of energy expenditure and fuel utilization in man: effects of body composition, age, sex, ethnicity and glucose tolerance in 916 subjects. Int J Obes Relat Metab Disord. 23 (7): 715-722. 1999.
- 15. World Health Organization. Report of a WHO Consultation on obesity: Preventing and managing the global epidemic. Geneva; 1999.

- Gallagher D, Allen A, Wang Z, Heymsfield SB, Krasnow N. Smaller organ tissue mass in the elderly fails to explain lower resting metabolic rate. Ann NY Acad Sci. 904: 449–455. 2000.
- 17. Wang Z, Heshka S, Heymsfield SB, Shen W, Gallagher D. A cellular-level approach to predicting resting energy expenditure across the adult years. Am J Clin Nutr. 81: 799–806. 2005.

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Risk factors for trimethoprim-sulfamethoxazoleresistance of E. Coli in children with urinary tract infection

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Abstract

Background: Urinary tract infections are common in children and E.Coli is the most common organism causing UTI. TMP-SMX is a frequently prescribed agent for UTI, but resistance level for this antimicrobial is gradually rising.

Methods: The aim of this study was to evaluate risk factors for development of trimethoprimsulfamethoxazole resistance of E.Coli in children with urinary tract infection with respect to age, gender, recent antimicrobial exposure and hospitalization. Between January 1, 2005, and December 31, 2010, a total of 397 isolates positive for E.Coli were evaluated retrospectively. We examined the relationships between trimethoprim-sulfamethoxazole resistance in E.Coli isolates and gender, age, recent antibiotic exposure, amoxicillin-clavulanate exposure and hospitalization. Univariate and multivariate logistic regression tests were applied for these variables.

Results: The mean age of the patients was 68.33±44.78 months. Of the 397 isolates, 50.6 % were resistant to TMP-SMX. Univariate analysis indicated that subjects who had received antibiotics in the previous 2 months were about 1.65 times more likely to have isolates resistant to TMP-SMX. Multivariate analyses indicated that subjects who had received antimicrobials were about 1.87 times more likely to have isolates resistant to TMP-SMX. Compared with children who had no hospital admissions in the previous 6 months, were more likely to have resistant isolates. Children under 4 years of age were more likely to have resistant isolates. Among specific antibiotic exposure, amoxicillin-clavulanate was found the main determinant of risk factor for TMP-SMX resistance.

Conclusion: In conclusions, recent antibiotic exposure, particularly amoxicillin-clavulanate, is a strong risk factor for the development trimethoprim-sulfamethoxazole-resistance of E.Coli in children with urinary tract infection, which limits the use of first-line antibiotics for UTI's in children.

Keywords: Urinary tract infection, Escherichia coli, trimethoprim–sulfamethoxazole resistance, children.

Introduction

Urinary tract infections are common clinical problem that affects 7 % of febrile children [1]. Young children with UTI are more prone to develop renal damage, and delayed treatment adds additional risks [2]; therefore, it is necessary to initiate treatment for UTIs before urine culture results are available [3]. However, a major concern is the identification of patients infected with a resistant microorganism in UTIs, because inappropriate empiric antibiotic treatment may cause high rates of treatment failures.

Previous studies in children have identified a variety of risk factors for antimicrobial resistance, such as contact with family members colonized with resistant bacteria [4], hospitalization [5], and antibiotic exposure [6]. The risk factors of Escherichia Coli antibiotic resistance in adults have been extensively studied and explained [6], but evidence for this causal relationship in community settings is limited in children.

The goal of this study study is to determine the risk factors for TMP-SMX resistance of Escherichia Coli isolated UTI in non-hospitalized children and to explore the relationships between age, gender, antibiotic exposure and hospitalization history.

Methods

This study is a retrospective study conducted between January 2005 and December 2010. We retrieved hospital electronic medical record (EMR) system using International Classification of Disease, Ninth Revision Codes. The EMR contains a patient's visit history, demographic information, laboratory data and medical history. The EMR was searched for children between 1 month and 15 years with a principal diagnosis of urinary tract infection and pyelonephritis.

All children had one of urinary symptoms such as dysuria, unexplained acute febrile fever, discomfort, or refusal to feed. The urine was collected thorough clean-catch method in children > 2years or bag-collection method in children < 2 years. A UTI was defined as 100 000 colony forming units (CFU) of a single microorganism. Microbiological analysis was performed by standard methods on cultures with bacteriuria using a panel of antimicrobial agents depending on the causative agent. Intermediate results were considered resistant results.

All E.Coli positive isolates whether UTI was complicated or uncomplicated were included to the study. The children who had isolates resistant to trimethoprim- sulfamethoxazole were considered as cases, and children who had TMP-SMX-sensitive isolates were considered as controls. Patients with malignancy, diabetes, immunodeficiency, central nervous system malformations, or genitourinary malformation and age < 1 month were excluded from the study. If the patients have been receiving prophylactic antibiotics, only antibiotic used during breakthrough UTIs were included.

Cases were defined as exposed to an antibiotic agent, which is defined as a risk factor if they were dispensed any antibiotics within 60 days of submission of the urine sample. An antimicrobial exposure was considered non-exposed, if the sample was obtained between 0 and 7 days of a urine culture. Information about antibiotic consumption before the diagnosis of UTI was obtained from patients' electronic charts, by questioning patients or their families. Hospitalization for any reason within 180 days before urine sampling date was defined as a risk factor. The patients were assembled into two groups according to their age, 1 month - 4

years, and 4-15 years, and age below 4 years was defined as a risk factor.

Data were analyzed by SPSS (version 20, Chicago, USA). The association between antimicrobial resistance and variables were first tested by using univariate logistic regression analysis. Subsequently, multivariate logistic regression analysis was performed to assess the association between antimicrobial resistance and the other factors. The terms included for regression analysis are age, gender, and antibiotic exposure within 60 days of UTI, and hospitalization within 180 days of UTI. OR's, 95% CI's, and p values are presented for variables.

Results

A total of 391 E.Coli isolates was obtained over the study period from 297 patients. The mean age of cases was 68.33 ± 44.78 months. Three hundred thirty eight (86.4 %) patients were female with the mean age 75.93 ± 42.60 months, 53 (13.6 %) were male with the mean age 19.87 ± 22.47 months. The mean age of females was significantly higher than males (mean 75.93 ± 42.60 versus 19.87 ± 22.47 months, p < 0.001). The number of patients aged 1 month to 4 years was 133 (34.0 %); the number of patients with age > 4 years was 258 (66.0 %). The number of patients hospitalized within 180 days of UTI was 28 (7.2 %). The patients characteristics is shown in table 1.

When all resistant isolates were examined as a proportion of the total number of isolates, the highest rates of resistance were found for amoxicillin (67.8 %), piperacillin (54.7 %), trimetho-Table 1. Patient characteristics

Variable	n	%
Gender		
Female	338	86.4
Male	53	13.6
Age		
1 mo \leq 4 years	133	34.0
> 4 years	258	66.0
Hospitalization		
No	363	92.8
Yes	28	7.2
Antibiotic Exposure		
No	303	77.5
Yes	88	22.5

prim-sulfamethoxazole (50.6 %), amoxicillinclavulanic acid (48.3 %), cefazolin (23.5 %), ceftriaxone (17.1 %), and the least rates resistance were found for meropenem (0.0 %), imipenem (0.5 %), amikacin (2.0 %), cefoperazone - sulbactam (3.6 %) and gentamicin (9.7 %) in E.Coli isolates. The rates of resistance to different antibiotics tested are reported in table 2.

Eighty- eight (22.5 %) patients received an antibiotic within 60 days of UTI for infections. The most commonly prescribed antibiotic was amoxicillin-clavulanic acid (7.9 %), prescription rates of cephalosporin's were as follows: cefixime, 3.3 %; cefaclor 1.3 %; cefuroxime, 2.6%; ceftriaxone, 0.8 %; cefdinir, 1.0 %; prescription rates of macrolides were 2.0 %, TMP-SMX prescription rate is 0.5 %. The prescription rates of amikacin and gentamicin are 0.5 %, 0.8 % respectively. Three hundred three (77.5 %) patients were not received any antibiotics. The rates of antibiotic prescriptions are reported in table 3.

Children who had received antibiotic treatment within 60 days of UTI were more likely to have resistant isolates than children who had not received

<i>Table 2. Rates of resistance to different antibiotics</i>	
tested against E. coli strains isolated from urinary	
tract infections	

	Resistant	Susceptible
	(%)	(%)
Amoxicillin	67.8	32.2
Amoxicillin- clavulanic acid	48.3	51.7
Trimethoprim- sulfamethoxazole	50.6	49.4
Amikacin	2.0	98.0
Gentamicin	9.7	90.3
Cefazoline	23.5	76.5
Cefotaxime	17.1	82.9
Cefepime	16.1	83.9
Cephoxitine	7.4	92.6
Cephoperazone- sulbactam	3.6	96.4
Ciprofloxacin	13.0	87.0
Levofloxacine	10.0	90.0
Imipenem	0.5	99.5
Meropenem	0.0	100
Piperacilin	54.7	45.3
Piperacine- Tazobactam	19.2	80.8

antibiotic treatment (p < 0.04 OR: 1.65 (95% (CI): 1.01-2.67)). The patients between 1 month- 4 years of age were more likely to have resistant bacteria (p < 0.02, OR: 1.63 (95% (CI): 1.06 - 2.49)). The other risk factor assessed was hospitalization within 180 days of UTI (p < 0.02, OR: 2.59 (95% (CI): 1.11 - 6.05). Univariate analysis showed that gender was not a risk factor for the development of TMP-SMX resistance (p= 0.22, OR: 1.44 (95% (CI): 0.80-2.59). The results of univariate analysis are shown in table 4.

In multivariate logistic regression analysis, the association between antibiotic exposure and TMP-SMX resistance was (p= 0.01 OR: 1.87, (95% (CI): 1.12 - 3.12)). The other association was age between 1 mo. - 4 years (p < 0.02, OR: 1.76, (95% (CI): 1.08 - 2.88). However, gender (p = 0.83, OR: 1.07 (95% (CI): 0.55 - 2.07), hospitalization (p= 0.09, OR: 2.81 (95% (CI): 0.88 - 4.95) were not associated with TMP-SMX resistance in multivariate logistic regression analysis. The results of multivariate analysis are shown in table 4.

The associations between antibiotic exposure and TMP-SMX resistance are shown in table 5. Amoxicillin-clavunate prescription is significantly associated with TMP-SMX resistance, OR: 3.03 (95 % (CI): 1.32 - 6.97, p= 0.009. It was found that exposure of cefixime, cefuroxime were not associated with TMP-SMX resistance. Due to small numbers, other antimicrobials used were not studied.

	n	%
None	303	77.5
Amoxicillin-Clavulanic acid	31	7.9
Ampicillin	7	1.8
Cefixime	13	3.3
Cefaclor	5	1.3
Cefuroxime	10	2.6
Macrolide	8	2.0
Trimethoprim- Sulfamethoxazole	2	0.5
Ceftriaxone	3	0.8
Cefdinir	4	1.0
Amikacin	2	0.5
Gentamicin	3	0.8
Total	391	100.0

	Univariate anal	ysis	Multivariate Analysis		
Variable	OR (CI 95 %)	р	(OR (CI 95%))	р	
Age					
>4 years	1		1		
1 mo-4 years	1.63 (1.06-2.49)	0.02*	1.76 (1.08-2.88)	0.02*	
Gender					
Female	1		1		
Male	1.44 (0.80- 2.59)	0.22	1.07(0.55-2.07)	0.83	
Hospitalization					
No	1		1		
Yes	2.59 (1.11-6.05)	0.02*	2.08 (0.88-4.95)	0.09	
Antibiotic Exposure					
No	1		1		
Yes	1.65(1.01-2.67)	0.04*	1.87 (1.12-3.12)	0.01*	

	Table 4.	Results	of lo	ogistic	regression	analyses
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* Statistically significant

Table 5. Prior antibiotic prescriptions and risk of trimethoprim-resistant E. coli UTIs

Antimicrobial Consumed	Category	R/S**	OR	95 % CI	р
Amoxicillin-clavulanate					
	no	175/185	1		
	yes	23/8	3.03	1.32-6.97	0.009*
Cefixime					
	no	191/187	1		
	yes	7/6	1.14	0.37-3.46	0.81
Cefuroxime					
	No	195/186	1		
	Yes	3/7	0.40	0.10-1.60	0.20

* Statistically significant; **R: resistant, S: Susceptible

Discussion

This study showed that a high number of E.Coli were resistant to first line antibiotics, trimethoprim-sulfamethoxazole, ampicillin and amoxicillin-clavunate, but resistances to carbapenems and amino-glycosides were found low. We found that infections with TMP-SMX resistant E.Coli were associated with prescriptions of amoxicillinclavunate in the previous 2-month of UTI. Gender was not associated with resistance while younger age was found to be a risk factor for the development of resistance. Children below 4 years of age had high range of resistant E.Coli. Hospitalization history within 6 mo. of UTI appears to be an independent risk factor for the development of resistance.

Trimethoprim-sulfamethoxazole (TMP-SMX) is one the most common prescribed antimicrobial for childhood UTIs [7], but the prevalence of resistance E.Coli is progressively increasing [8]. Since early 1990s, in some European Countries, TMP-SMX resistance rate was reported about 10 %, but within 10 years period, it increased to 17% [9]. In a prospective study in Turkey TMP-SMX resistance of E.Coli was found about 60% [10]. Our study demonstrated that resistance level to TMP-SMX was 50.6 %. Although not every patient infected with a resistant microorganism fail treatment, it was shown that patients with in vitro resistant isolates to TMP-SMX had high rates of treatment failures [11]. Our results suggest that the use of TMP-SMX as a single agent for the empirical treatment of UTIs do not cover E.Coli.

In adults a wide range of antibiotic selection is applicable for UTIs; however, there is a restricted choice of first-line antimicrobials in UTIs due to resistant organism in children. A combination of empirical ampicillin and amino-glycosides was suggested for UTIs in children [12]. Other authors suggested amoxicillin-clavunate or cefuroxime [13], or cefixime [14] for empirical treatment of community acquired UTIs. Our result suggests that resistance rates for amino-glycosides are relatively low; therefore, amino-glycosides can be used for empirical treatment of UTIs.

Although antibiotics have been effective in control of infectious diseases, extensive prescription and use of antibiotics caused the development and spread of antimicrobial resistance [15]. The univariate analysis of this study demonstrates that antibiotic exposure within 60 days of a UTI is strongly associated with TMP-SMX resistance among pediatric outpatients. In adult patients an association between excessive and/or inappropriate antibiotic use and resistance in UTI has been demonstrated [16]. In children, prolonged use of antibiotics such as for antibiotic prophylaxis was also associated with increased risk of resistant infections [17]. Policies against use of antimicrobials prescription have been successful; however, three are still high rates of antimicrobial prescription rates [18]. Our study showed that prescription rates of antibiotics were 22.5 % in children. In pediatric age group, acute bacterial infections, such as acute otitis media, sinusitis is common and antibiotics are generally prescribed [19]. However, guidelines and studies suggest limited use of antibiotics for these clinical conditions and short course of antibiotics for more severe infections, such as lower respiratory infections [20]. Therefore, it is necessary to restrict antimicrobial use to combat antimicrobial resistance.

Trimethoprim-sulfamethoxazole is considered an indicator first line agent that resistance to TMP-SMX was associated with resistance to other, pharmacologically unrelated agents, such as amoxicillin and first-generation cephalosporin's [21]. Thus, the main risk factors associated with TMP resistance would be associated with other agents. Our result showed that recent use of amoxicillin-clavunate increases the risk of TMP-SMX resistance development about 3 times more (OR: 3.03, p<0.009). It has been showed that use of amoxicillin-clavunate resulted in a high rate of ampicillin and sulfamethoxazole resistance, and is postulated that a partial cross-resistance mechanism played a role for the development of resistance [22]. The correlations between amoxicillin-clavunate use and TMP-SMX resistance suggested a linked resistance to both antibiotics. Transports of a linked resistance to these antibiotics via transmissible plasmids have been documented in fecal E.Coli [23]. The results of present study suggest that the same mechanism is involved in urinary E.Coli infections.

Although infections with resistant enterobacteriaceae in children have been reported more common in males [24], our study showed that resistant rates regarding to sex was insignificant. This study demonstrated that antibiotic resistance was more common in younger age. The odds for resistant rates in children below 4 years age was 1.643 (1.06-2.49). Studies have shown that the resistance rates of E.Coli to TMP-SMX were found higher in younger infants [25, 26]. This high rate of resistance was attributed that younger aged children had a dysfunctional elimination of urine [27] that might lead to recurrent UTI, which in turn may increase probability of frequent antibiotic prescription, and that the younger age groups have high rates of respiratory tract infections [28], this may provide the rationale used by some physicians to prescribe multiple and prolonged courses of antibiotics in this age group. Another possible explanation is that the development of antimicrobial resistance in younger children was associated with diapering because resistant colonization of E.Coli was found to be high in diapered children; this was attributed to higher prevalence of fecal colonization with multi-resistance E.Coli [21].

It is well known that hospitalization is a major risk factor for the development of antimicrobial resistance [29]. We found that admission to hospital within 6 mo. of UTI increases the risk of resistance assessed by univariate analysis. However, in multivariate testing hospitalization did not contribute the development of resistance. This suggests that hospitalization is independent risk factor, and suggest spread of resistance within closed settings.

We have some limitations. We studied health children without major problems; our results are not applicable to hospitalized children with complex problems or those cared in intensive care unit. Another limitation is that data on antimicrobial drug exposure were limited to information from outside our institution and limited to parenteral reporting; therefore, we could not be able to define dose-response relationships for antimicrobial-resistant infections, because mathematical models indicate when a critical level of drug consumption is reached, the prevalence of resistant bacteria will rise to significant levels as antibiotic exposure increases, and any antimicrobial drug reduces colonization with susceptible bacteria this in turn leads to an increased the probability of colonization by resistant bacteria [30].

Conclusions

Recent antibiotic exposure is a strong risk factor for the development of antimicrobial resistance for E.Coli UTIs in children. The data in this study allow some general conclusions to be made. First-line antibiotics such as amoxicillin and TMP-SMX should no longer be considered first-line agents in the treatment of most pediatric UTIs. Our results suggest that inappropriate use of antimicrobials should be limited in order to reduce the development of antimicrobial resistance.

Further prospective studies are required to search the similar data to evaluate this relationship in children. Frequent surveillance is necessary to observe antimicrobial resistance patterns. We suggest that the policies for the choice of antibiotic regimes for uncomplicated UTI in children should be reviewed every 2-3 years.

References

- 1. Shaikh N, Morone NE, Bost JE, Farrell MH. Prevalence of urinary tract infection in childhood: a metaanalysis. Pediatr Infect Dis J. 2008;27:302-308.
- 2. Smellie JM, Normand IC. Urinary infections in children 1985. Postgrad Med J. 1985;61:895-905.
- 3. Coulthard MG, Verber I, Jani JC et al. Can prompt treatment of childhood UTI prevent kidney scarring? Pediatr Nephrol. 2009;24:2059-2063.
- Lietzau S, Raum E, von Baum H, Marre R, Brenner H. Household contacts were key factor for children's colonization with resistant Escherichia coli in community setting. J Clin Epidemiol. 2007;60:1149-1155.
- Steinke DT, Seaton RA, Phillips G, MacDonald TM, Davey PG. Prior trimethoprim use and trimethoprimresistant urinary tract infection: a nested case-control study with multivariate analysis for other risk factors. J Antimicrob Chemother. 2001;47:781-787.

- 6. Hillier S, Roberts Z, Dunstan F, Butler C, Howard A, Palmer S. Prior antibiotics and risk of antibioticresistant community-acquired urinary tract infection: a case-control study. J Antimicrob Chemother. 2007;60:92-99.
- 7. Copp HL, Shapiro DJ, Hersh AL. National ambulatory antibiotic prescribing patterns for pediatric urinary tract infection, 1998-2007. Pediatrics. 2011;127:1027-1033.
- 8. Zhanel GG, Hisanaga TL, Laing NM et al. Antibiotic resistance in outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). Int J Antimicrob Agents. 2005;26:380-388.
- 9. Abelson Storby K, Osterlund A, Kahlmeter G. Antimicrobial resistance in Escherichia coli in urine samples from children and adults: a 12 year analysis. Acta Paediatr. 2004;93:487-491.
- 10. Yuksel S, Ozturk B, Kavaz A et al. Antibiotic resistance of urinary tract pathogens and evaluation of empirical treatment in Turkish children with urinary tract infections. Int J Antimicrob Agents. 2006;28:413-416.
- 11. Prelog M, Schiefecker D, Fille M, Wurzner R, Brunner A, Zimmerhackl LB. Febrile urinary tract infection in children: ampicillin and trimethoprim insufficient as empirical mono-therapy. Pediatr Nephrol. 2008;23:597-602.
- 12. Haller M, Brandis M, Berner R. Antibiotic resistance of urinary tract pathogens and rationale for empirical intravenous therapy. Pediatr Nephrol. 2004;19:982-986.
- 13. Prais D, Straussberg R, Avitzur Y, Nussinovitch M, Harel L, Amir J. Bacterial susceptibility to oral antibiotics in community acquired urinary tract infection. Arch Dis Child. 2003;88:215-218.
- 14. Hoberman A, Wald ER, Hickey RW et al. Oral versus initial intravenous therapy for urinary tract infections in young febrile children. Pediatrics. 1999;104:79-86.
- 15. Schaeffer AJ. Urinary tract infections: antimicrobial resistance. Curr Opin Urol. 2000;10:23-24.
- 16. Fishman N. Antimicrobial stewardship. Am J Infect Control. 2006;34:S55-63; discussion S64-73.
- Conway PH, Cnaan A, Zaoutis T, Henry BV, Grundmeier RW, Keren R. Recurrent urinary tract infections in children: risk factors and association with prophylactic antimicrobials. JAMA. 2007;298:179-186.

- 18. Sommet A, Sermet C, Boelle PY, Tafflet M, Bernede C, Guillemot D. No significant decrease in antibiotic use from 1992 to 2000, in the French community. J Antimicrob Chemother. 2004;54:524-528.
- 19. Thompson PL, Spyridis N, Sharland M et al. Changes in clinical indications for community antibiotic prescribing for children in the UK from 1996 to 2006: will the new NICE prescribing guidance on upper respiratory tract infections just be ignored? Arch Dis Child. 2009;94:337-340.
- 20. Arroll B. Antibiotics for upper respiratory tract infections: an overview of Cochrane reviews. Respir Med. 2005;99:255-261.
- 21. Reves RR, Fong M, Pickering LK, Bartlett Ar, Alvarez M, Murray BE. Risk factors for fecal colonization with trimethoprim-resistant and multiresistant Escherichia coli among children in day-care centers in Houston, Texas. Antimicrob Agents Chemother. 1990;34:1429-1434.
- 22. Kahlmeter G, Menday P, Cars O. Non-hospital antimicrobial usage and resistance in community-acquired Escherichia coli urinary tract infection. J Antimicrob Chemother. 2003;52:1005-1010.
- 23. Gulay Z, Bicmen M, Amyes SG, Yulug N. Betalactamase patterns and betalactam/clavulanic acid resistance in Escherichia coli isolated from fecal samples from healthy volunteers. J Chemother. 2000;12:208-215.
- 24. Bitsori M, Maraki S, Kalmanti M, Galanakis E. Resistance against broad-spectrum beta-lactams among uropathogens in children. Pediatr Nephrol. 2009;24:2381-2386.
- 25. Allen UD, MacDonald N, Fuite L, Chan F, Stephens D. Risk factors for resistance to "first-line" antimicrobials among urinary tract isolates of Escherichia coli in children. CMAJ. 1999;160:1436-1440.
- Ismaili K, Lolin K, Damry N, Alexander M, Lepage P, Hall M. Febrile urinary tract infections in 0- to 3-month-old infants: a prospective follow-up study. J Pediatr. 2011;158:91-94.
- 27. Mazzola BL, von Vigier RO, Marchand S, Tonz M, Bianchetti MG. Behavioral and functional abnormalities linked with recurrent urinary tract infections in girls. J Nephrol. 2003;16:133-138.
- 28. Wald ER, Guerra N, Byers C. Frequency and severity of infections in day care: three-year follow-up. J Pediatr. 1991;118:509-514.

- 29. Colodner R, Rock W, Chazan B et al. Risk factors for the development of extended-spectrum beta-lactamase-producing bacteria in nonhospitalized patients. Eur J Clin Microbiol Infect Dis. 2004;23:163-167.
- 30. Austin DJ, Anderson RM. Studies of antibiotic resistance within the patient, hospitals and the community using simple mathematical models. Philos Trans R Soc Lond B Biol Sci. 1999;354:721-738.

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Serum cytokine levels in asymptomatic Brucella seropositive individuals

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Abstract

Brucellosis is still prevalent in many countries. Despite a great deal of understanding in its immunopathogenesis, a vaccine for humans is not yet available. In this report, we compared serum proinflammatory cytokines, tumor necrosis factor (TNF)- α , interleukin (IL)-1 β and IL-6, between brucellosis patients, their asymptomatic seropositive household members and controls. Twentysix symptomatic, 10 asymptomatic and 16 control cases were included. Serum cytokine levels were determined using a chemiluminescent immunoassay kit. We determined elevated TNF- α levels in brucellosis patients, while all three cytokine levels were similar between asymptomatic and control groups. Our results suggest that asymptomatic seropositive individuals have indeed successfully controlled their Brucella exposure. Further studies to delineate Brucella specific T cell response in these subjects will be valuable for designing a vaccine for humans.

Key words: Brucellosis, proinflammatory cytokines, tumor necrosis factor, Brucella exposure.

Introduction

Brucellosis is a common zoonotic infection. Humans acquire Brucella from domestic livestock via direct contact, inhalation or ingestion. Although there is an effective vaccine for animal control, animal brucellosis is still prevalent in developing countries due to inadequate livestock vaccination. Consequently, human brucellosis, characterized by fever, malaise, fatigue, anorexia, headache and back pain, is still prevalent (1, 2).

Usually only symptomatic Brucella infections come to clinical attention and most of the time they

can be cured by appropriate kind and duration of antibiotic therapy. One uncertainty about the management of Brucella infection is whether family members of the index case should be screened and treated, if tested seropositive. As most of the time etiology appears to be the ingestion of contaminated food, it is natural to presume that family members with similar dietary habits are exposed to the same infectious source. In a previous study, we found that screening of family members living in the same household of the index brucellosis case identifies seropositive individuals with or without symptoms (3). Although it is certainly beneficial for symptomatic cases to be treated, we found out that none of the asymptomatic seropositive cases developed overt brucellosis within 6-12 months of follow-up. Thus, arguing for successful immune control of Brucella exposure by these individuals.

To take this observation further and in an effort to detect occult latent infection, we compared serum cytokine patterns of asymptomatic seropositive cases with symptomatic cases and controls. The quintessential proinflammatory cytokines, tumor necrosis factor (TNF)- α , interleukin (IL)-1 β and IL-6, are mainly secreted by macrophages (4). They are mostly elevated during acute and chronic Brucella infection and regress to baseline levels with successful treatment (5, 6). Our results indicate and further support the notion from our previous study that asymptomatic seropositive individuals successfully controlled Brucella exposure.

Materials and method

Patients

We studied 26 symptomatic, 10 asymptomatic and 16 control cases. All cases were tested for brucellosis by slide agglutination (Rose Bengal) test. Positive samples were further tested by tube agglutination (Wright) test using Brucella abortus antigen to determine the titer. Patient characteristics and clinical definitions are described in detail in a previous paper (3). Briefly, symptomatic cases are those with signs and symptoms of brucellosis with tube agglutination titers $\geq 1:80$. All cases were considered subacute as the duration of symptoms was 8-52 weeks (7). Asymptomatic cases are those from the same household with tube agglutination titers $\geq 1:80$ but without signs and symptoms of brucellosis. Controls were seronegative members of the same household.

This study was approved by the local ethics committee of Istanbul University, Cerrahpasa Medical Faculty (No: 31776). All participants gave informed consent.

Measurement of serum cytokine levels

After an overnight fast, venous blood samples were drawn into tubes without additives, early in the morning. After one hour of upright incubation at room temperature, tubes were spun at 400g and serum was collected and frozen at -80oC until further analysis. Concentrations of free cytokines TNF- α , IL-1 β , and IL-6 were determined using a commercially available chemiluminescent immunoassay kit according to the manufacturer's instructions (Immulite®, DPC, Los Angeles, CA, USA).

Statistical analysis

Cytokine levels between the groups were compared using one-way ANOVA. Scheffe's post-hoc test was used. p<0.05 is considered as significant.

Results

Twenty-six symptomatic, 10 asymptomatic and 16 control cases were included in this study. Patients' age ranged from 12 to 66 (median: 35),

Table 1. Mean plasma cytokine levels of 3 groups

12 to 49 (median: 25) and 3 to 48 (median: 14) years in symptomatic, asymptomatic and control group, respectively. Seventy four percent of symptomatic, 40% of asymptomatic and 50% of control group were male.

Serum cytokine levels of the study groups are shown in the Table. Mean serum levels of TNF- α were significantly higher in symptomatic group (24.4±21.5 pg/mL, mean±SD) compared to asymptomatic (11.3±6.2, p=0.014) and control groups (12.1±6.6, p=0.013). There was no statistically significant difference between TNF- α levels of asymptomatic cases and controls. Individual TNF- α levels are shown in the Figure. There were no statistically significant differences for serum IL-1 β and IL-6 levels between the groups. Thus, asymptomatic seropositive cases and controls had similar serum levels of cytokines TNF- α , IL-1 β and IL-6.

Discussion

In this study, we compared serum cytokine levels of TNF- α , IL-1 β and IL-6 between patients with symptomatic brucellosis, their asymptoma-



Figure 1. TNF- α levels. Serum TNF- α levels of individual cases within the study groups are shown. Bars indicate average for each study group. p<0.05 when symptomatic group is compared to asymptomatic or control groups

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	Symptomatic			Asymptomatic			Control		
	mean±SD ^b	median	range	mean±SD	median	range	mean±SD	median	range
TNF-α	24.4±21.5°	15.7	6–92.2	11.3±6.2	10.8	4-21.2	12.1±6.6	8.7	4.7-27.1
IL-1β	8.2±13	5	5-70.1	5±0	5	5-5	5.6±2.7	5	4–15.7
IL-6	19.4±46	6.5	2–219	6.2±9.1	2.7	2-31.5	15.1±33.5	3	2-31

^a all values are in pg/mL

^b standard deviation (SD)

^c p<0.05 compared to asymptomatic and control cases

tic household relatives with serologic evidence of Brucella exposure and controls. TNF- α was found to be significantly higher in symptomatic patients. Asymptomatic seropositive and control individuals had similar serum levels of TNF- α , IL-1 β and IL-6.

Animal studies have demonstrated that an orchestrated effort by innate and adaptive arms of immune response is required for elimination of Brucella challenge (8). TNF- α release by macrophages and interferon- γ release by T helper-1 polarized CD4 cells appear to play crucial roles in controlling Brucella infection (9). Serum levels of several cytokines were studied in humans with brucellosis to understand its immunopathogenesis (5). Increase in one or more of the cytokines TNF- α , IL-1 β and IL-6 during acute, subacute or chronic infection with eventual regression to baseline levels after successful treatment have been shown in human subjects (7, 10, 11). Such elevated levels are probably due to ongoing struggle between Brucella and its host. Accordingly, we also found elevated levels of TNF- α in subjects with brucellosis compared to asymptomatic seropositive individuals and controls.

To establish infection, Brucella must evade immune response and proliferate within macrophages like other intracellular bacteria (6, 9, 12). Thus, patients with brucellosis represent a failed attempt by the immune system to eradicate Brucella. We had found in a previous study that screening same household members of the index brucellosis case identifies seropositive but asymptomatic individuals (3). Such individuals may either have a latent infection or have successfully controlled the exposure. We did not detect any of them developing symptomatic disease in 6 to 12 months of follow-up arguing for successful control of exposure. In this study, we provide further evidence for successful immune control of Brucella exposure by detecting essentially similar levels of proinflammatory cytokines in asymptomatic seropositive individuals compared to controls. Our study provides further support to the clinical approach that these individuals do not need immediate treatment but be followed and treated when and if they become symptomatic.

The exact determinants of resolution of Brucella exposure are not well-understood (9, 13). Seropositive asymptomatic individuals provide a unique opportunity for understanding successful immune control of Brucella exposure. Further studies to delineate antigenic epitopes recognized by CD4 and CD8 lymphocytes in these individuals will be valuable for designing a subunit vaccine for humans.

Referances

- Young EJ (2005) Brucella Species. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and Practice of Infectious Diseases. 6th ed. Philadelphia: ELSE-VIER. p. 2670-5.
- 2. Solera J (2010) Update on brucellosis: therapeutic challenges. Int J Antimicrob Agents 36 Suppl 1:S18-20. Epub 2010/08/10.
- 3. Tabak F, Hakko E, Mete B, Ozaras R, Mert A, Ozturk R (2008) Is family screening necessary in brucellosis? Infection 36(6):575-7. Epub 2008/11/18.
- 4. Janeway CA, Jr., Medzhitov R (2002) Innate immune recognition. Annu Rev Immunol 20:197-216. Epub 2002/02/28.
- Dornand J, Gross A, Lafont V, Liautard J, Oliaro J, Liautard JP (2002) The innate immune response against Brucella in humans. Vet Microbiol 90(1-4):383-94. Epub 2002/11/05.
- 6. Giambartolomei GH, Delpino MV, Cahanovich ME, Wallach JC, Baldi PC, Velikovsky CA, et al. (2002) Diminished production of T helper 1 cytokines correlates with T cell unresponsiveness to Brucella cytoplasmic proteins in chronic human brucellosis. J Infect Dis 186(2):252-9. Epub 2002/07/23.
- 7. Akbulut H, Celik I, Akbulut A. Cytokine levels in patients with brucellosis and their relations with the treatment (2007). Indian J Med Microbiol 25(4):387-90. Epub 2007/12/19.
- Moreno-Lafont MC, Lopez-Santiago R, Zumaran-Cuellar E, Paredes-Cervantes V, Lopez-Merino A, Estrada-Aguilera A, et al. (2002) Antigen-specific activation and proliferation of CD4+ and CD8+ T lymphocytes from brucellosis patients. Trans R Soc Trop Med Hyg 96(3):340-7. Epub 2002/08/15.
- 9. Yingst S, Hoover DL (2003) T cell immunity to brucellosis. Crit Rev Microbiol 29(4):313-31. Epub 2003/11/26.
- 10. Refik M, Mehmet N, Durmaz R, Ersoy Y (2004) Cytokine profile and nitric oxide levels in sera from patients with brucellosis. Braz J Med Biol Res 37(11):1659-63. Epub 2004/11/02.

- 11. Rodriguez-Zapata M, Matias MJ, Prieto A, Jonde MA, Monserrat J, Sanchez L, et al. (2010) Human brucellosis is characterized by an intense Th1 profile associated with a defective monocyte function. Infect Immun 78(7):3272-9. Epub 2010/04/21.
- Barrionuevo P, Delpino MV, Velasquez LN, Garcia Samartino C, Coria LM, Ibanez AE, et al. (2011) Brucella abortus inhibits IFN-gamma-induced FcgammaRI expression and FcgammaRI-restricted phagocytosis via toll-like receptor 2 on human monocytes/macrophages. Microbes Infect 13(3):239-50. Epub 2010/11/13.
- 13. Skendros P, Pappas G, Boura P. Cell-mediated immunity in human brucellosis. (2011) Microbes Infect 13(2):134-42. Epub 2010/11/03.

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Sexual attitudes and sexual behaviours toward sexually transmitted diseases among Chinese male migrant workers

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Abstract

Background: China is facing a critical challenge in combating rapid and widespread increase in human immunodeficiency virus (HIV) / sexually transmitted infections (STIs). Rural-to-urban migration plays a crucial role in shifting the HIV/ STIs epidemic in China. The purpose of this study was to assess the prevalence of sexual behaviours, the correlation between sexual attitudes and sexual behaviours, and factors associated with the sexual behaviours among male migrant workers (MMWs) in China.

Methods: Using the cluster sampling method, we investigated the sexual attitudes toward AIDS and other sexually transmitted diseases and the sexual behaviours of Chinese MMWs, the relationship between their sexual attitudes and sexual behaviours, and relevant factors affecting the sexual behaviours.

Results: Among married migrants, 26.8% had one extra-marital sex partner, 26.8% had more than two sex partners, and 7.7% and 6.0% used condoms during every sexual intercourse with a married partner and with an illegal partner, respectively. Among unmarried migrants, 62.2% had premarital sex, 42.1% had more than two sex partners, and only 8.6% used condoms during every sexual intercourse. Bivariate regression analyses found that factors significantly associated with premarital sex in unmarried MMWs included their attitude of premarital sex, age and family income; and their attitude of having multiple sexual partners was significantly associated with extra-marital sex in married MMWs.

Conclusion: This study provides important new information for understanding the sexual behaviours in China and suggests that there is an urgent need for comprehensive prevention programs that should include more efforts on sex education and condom promotion among MMWs in China.

Keywords: China, migrant workers, acquired immunodeficiency disease (AIDS), sexual attitudes, sexual behaviours.

Introduction

The increasing spread of sexually transmitted diseases (STDs) including AIDS has become an international public health issue. Over the past decade, migrant workers have played an increasingly significant role in the construction and development of Chinese cities during its rapid economic growth and urbanization. Migrant workers are ex-farmers who leave their homes in the countryside for work in cities. The majority of migrant workers are male. They move from one place to another and usually have high-risk sexual behaviours, which is an important factor in the spread of AIDS. (1-3)

In many countries, geographic mobility, particularly male rural-to-urban economic migration, is a key driver of increased HIV and STIs transmission. (4-8) In China, in recent years, infection through sexual transmission has been growing rapidly. Sexually transmitted cases have increased from 7.2% in 2002 to 43.6% in total infections by the end of 2005.(9) In 2007, heterosexual transmission of HIV has accounted for 44.7% of new infection cases and has become the dominant mode of HIV transmission.(10) Male migrant workers (MMWs) may be vulnerable to infection for many reasons including separation from spouses and family, alcohol use, low perceived vulnerability to HIV/STIs infection, limited access to health care and low levels of education. (11-14)

According to a former definition, (15) the concept of sex behaviours can be understood in both broad and narrow senses. Broadly speaking, sex behaviours refer to the phenomenon that human individuals consciously touch sexually sensitive body parts of theirs or their sexual partner(s), such as hugs, kisses, and caress, etc. By contrast, sex behaviours in its narrow sense refer exclusively to contacts of sex organs. Sex behaviours in the present study were confined to its narrow sense.

The term "sexual attitudes" refers to the subjective understandings of sex. They are relatively stable views and appraisals of sex, including sexual attitudes of an individual and general appraisal, attitudes and perspectives of a particular society concerning sex. (16) The term "sexual attitudes" in the present study means the views, attitudes, and appraisals of MMWs towards sexual behaviours.

Research has shown that, among the factors influencing the epidemic of STDs and AIDS, sexual behaviours (which are usually controlled by the sexual attitudes that have been formed in a person's mindset for a long time) play an important role. Sexual behaviours are driven by sexual conception. Incorrect knowledge and attitudes toward certain sexual issues will lead people to practice improper sexual behaviours. (17,18)

China is facing a critical challenge in fighting rapid and widespread increase in HIV/AIDS cases. In 2007, the Chinese government along with the World Health Organization (WHO) and the Joint United Nations Programme on HIV/AIDS (UNAIDS) estimated that 700,000 people were living with HIV in China, including 85,000 AIDS patients. Meanwhile, a number of HIV prevention interventions have been conducted, but most studies have been concentrated in South and Southwest China, mainly targeting injection drug users and female sex workers.(19-21) Few studies have been conducted among MMWs in central and western regions, particularly sexually active male migrant workers.

Therefore, this study aimed to explore the sexual attitudes and prevalence of sexually risky behaviours (including premarital sex, multiple sexual partners, commercial sex, and unprotected sex), the relationship between sexual attitudes and sexual behaviours and the factors influencing their sexual behaviours among male construction migrant workers in China, propose recommendations for future interventions, and provide evidence for implementing education strategies to prevent STIs and AIDS among MMWs in China.

Materials and methods

A cross-sectional survey was conducted among 936 rural-to-urban male migrants in ten large Chinese cities: Xi'an, Nanjing, Wuhan, Kaifeng Chengdu, Lanzhou, Xining, Taiyuan, Changsha, and Jinan, which are the capitals of Shaanxi, Jiangsu, Hubei, Henan, Sichuan, Gansu, Qinghai, Shanxi, Hunan, and Shandong provinces, respectively. Xi'an, Nanjing, Wuhan, Kaifeng Chengdu, Lanzhou, Xining, Taiyuan, Changsha, and Jinan were among the Chinese administrative regions that reported the highest incidence of sexually transmitted diseases (22-25) and also among the Chinese cities with large numbers of rural-to-urban migrants.(26-27)

This study first used stratified random sampling in ten provinces in China, including Shaanxi, Jiangsu, Hubei, Henan, Sichuan, Gansu, Qinghai, Shanxi, Hunan, and Shandong. Next, the purposive sampling method was employed to select ten provincial capital cities from each province. Thirdly, quota-sampling was used to choose one construction site from each city. The MMWs who met the inclusion criteria were all enrolled on a voluntary basis in the selected construction site.

The study was approved by the Ethical Review Committee of the First Affiliated Hospital of Xi'an Jiaotong University. We also obtained consent from the centers for disease control and prevention of each city and the health management of the each construction site involved. Informed consent was required from each subject prior to their participation of the survey. Every migrant worker filled out the questionnaire in a separate room in the absence of interference from others. The participants were informed of the objectives of the study, their confidentiality and their right to refuse to participate in the study. Each subject participated in the study was given a small gift. The study was conducted from June 2010 to Oct 2011.

Inclusion criteria and exclusion criteria

MMWs who had been working in the cities for more than three consecutive months and without impairment of intelligence or cognition, as evaluated by their ability to respond to the questions, were eligible for the study.

Structure of questionnaire

The survey questionnaire was designed in reference to the questionnaires of sexual knowledge, sexual attitudes, and sexual behaviours of the WHO, the Questionnaire of Demand Assessment of Information and Media Materials for AIDS Prevention in the Countryside written by the Health Education Institute of China Disease Prevention and Control Center, as well as other relevant documents and literature. The questionnaire comprised general epidemiological data and questions about sexual attitudes and sexual behaviours. A five-scale scoring method was applied: absolutely agree, agree, it does not matter to me, disagree, and absolutely disagree. The five types of answers corresponded to scores of 5, 4, 3, 2, and 1, respectively, with a total possible score of 25. An overall score of above 18 was regarded as open and tolerant; a score between 9 and 18 was regarded as relatively open and tolerant; and a score below 9 was regarded as healthy.

Reliability and validity of the questionnaire

The questionnaires of sex knowledge, sex attitudes, and sex behaviours of the WHO in the English language have been widely used internationally and have high validity and reliability. The questionnaire was first translated into Chinese by an English-language expert, and the Chinese version was translated back into English by another English-language expert. After the two experts reached complete agreement with the translation of the questionnaire, we revised the questionnaire to be context-specific to China based on a review of the relevant literature and suggestions made by experts from social psychological, epidemiological, nursing, and sexual health professions. After the revision was made, the validity of the questionnaire was assessed by five relevant experts including two AIDS experts, one psychosocial expert, one epidemiologist, and one language expert. After all of the experts agreed on the questionnaire, the content validity index (CVI) was calculated. A CVI ≥ 0.80 is generally regarded as acceptable. The questionnaire used in this survey had a CVI=0.86. Before the formal survey was conducted, a pilot trial was conducted on 30 MMWs from the Shaanxi province who met the inclusion criteria. The pilot results showed that the internal Cronbach's alpha of the questionnaire was 0.87. Therefore, the reliability and validity of the questionnaire were both acceptable.

Methods

At the beginning of the survey, participants were informed of the aim and major contents of the survey to reduce errors or bias as much as possible ("self-protection" bias). After receiving informed consent from the migrant workers, we distributed the questionnaires to them on the spot.

Participants were asked to complete a self-administered anonymous questionnaire in a separate room or private space at the workplace or a nearby community location convenient to participants. When the participants were answering the questionnaire, their wives or girlfriends were asked not to accompany them. Standardized instructions were given to the participants. The questionnaire, which was administered in Mandarin Chinese, took 20-30min to complete. Reading assistance was provided to a few participants with limited literacy. Then, the questionnaires were anonymously filled in by the MMWs and returned immediately after they were completed.

Statistics

All statistical analyses were performed using SPSS software, version 10.01 (SPSS, Chicago, IL, USA). Frequencies and percentage ratios were used to evaluate the obtained data. Spearman correlation analysis was used to test for the relationship between sexual attitudes and sexual behaviours. Bivariate logistic regression analysis was used to test for independent factors associated with sexual behaviours. P<0.05 was considered statistically significant.

Results

A total of 1,188 questionnaires were distributed and 965 were returned. The response rate was 81.2%. Among them, 936 questionnaires were accepted; the acceptance rate was 97.0%. There were 29 rejected questionnaires due to their incompleteness or identical answers between the questionnaires.

General demographic characteristics of the survey subjects

The participants were from Xi'an (182), Nanjing (129), Wuhan (118), Kaifeng (102), Chengdu (93), Lanzhou (86), Xining (73), Taiyuan (69), Changsha (57), and Jinan (56), The mean age of the participants was 33 +/-10 years (range 15-60 years). Sixty-five percent (608) of the participants were younger than 40 years; 73.9% (691) of the participants were married; 75.6% (708) of the participants had an educational level lower than junior high school; and 54.7% (512) of them had a yearly family income of 5,000-10,000 yuan. Furthermore, 85.0% (796) of the families had to pay their medical bills at their own expense; 67.2% (629) of the families relied on the MMWs as the main breadwinners; and 15.1% (141) of them were previously accompanied by their wives or girlfriends when they worked in the cities; 16.2% of them (151) had their wives or girlfriends accompanying them at the time of the survey. Overall, 70.1% of them spent their spare time sleeping and 52.3% chatting, 7.7% went to Karaoke and Disco, and 10.0% went to beauty salons for commercial sex services.

Sexual attitudes of the survey subjects

As shown in Table 1, 19.8% of the MMWs regarded premarital sex as acceptable; 12.7% accepted extra-marital sex; 10.1% accepted multi-partner sex; and 11.3% accepted commercial sex.

Sexual behaviours of the respondents

Table 2 presents the types and number of sex partner(s) of the respondents and the frequency and knowledge of condom use by them. 26.8% of the married men had more than two sex partners; 43.1% did not know how to use condoms appropriately; 53.9% did not prefer to use condoms when they had sexual intercourse; 47.0% never used condoms when having sex with their wives. 53.5% never used condoms when having extra-marital sex, 17.3% used alcohol, and 7.6%

used drugs. The usage of condoms was significantly less frequent among married MMWs who used drugs (0%) or used alcohol (18.7%) than those who did not (above 29.8%). 82.9% of unmarried men did not use condoms during their first sexual intercourse; 42.1% had more than two sex partners; 67.0% did not know or were uncertain about how to use condom correctly.

Relationship between sexual attitudes and sexual behaviours among the subjects

As shown in Table 3, a significant positive correlation was found between sexual attitudes and extra-marital sex status in married men, between sexual attitudes and premarital sex status, and between the number of sex partners and sexual attitudes in unmarried men.

Factors affecting sexual behaviours

Table 4 shows a bivariate logistic analysis of factors related to premarital sex of unmarried male migrants. Factors related to premarital sex of unmarried male migrants included their attitudes of premarital sex (OR = 0.26, 95% CI 0.11-0.56, p < 0.01). Unmarried workers with a higher level of attitudes of premarital sex were more likely to have premarital sex than those with a lesser level. Age was related to premarital sex of unmarried male migrants. Younger unmarried male migrants were less likely to have premarital sex (OR = 0.33, 95% CI 0.15–0.73, p < 0.05. Yearly family income also related to premarital sex of unmarried male migrants. No obvious difference regarding premarital sex among unmarried male migrants was noted between middle-income and low-income.

Table 5 shows a bivariate logistic analysis of factors related to extra-marital sex of married male migrants. Factors related to extra-marital sex of married male migrants included their attitudes of multi-partner sex (OR = 0.25, 95% CI 0.15–0.45, p < 0.001). Married workers with a higher level of acceptance of multi-partner sex were more likely to have extra-marital sex than those with a lower level.

Discussion

This survey was conducted in ten Chinese cities with high STI/AIDS prevalence and large num-

bers of rural-to-urban migrants to examine sexual behaviours and condom use among MMWs. Our findings reveal behaviours placing MMWs at high risk for HIV / STI infection.

Our findings found that less than 20% of MMWs thought premarital sex, extra-marital sex, multi-partner sex, prostitution and other commercial sex services were acceptable, but the prevalence of premarital, extra-marital and multi-partner sexual behaviours seemed high. Among married migrants, 26.8% had one extramarital sex partner, 26.8% had more than two sex partners. Among unmarried migrants, 62.2% had premarital sex, 42.1% had over two sex partners. This disparity may be partially due to self-report bias, and partially due to Chinese culture and tradition, for it is a taboo to talk freely and openly about sex. (28) This belief is especially deep-rooted in people from the countryside compared with city dwellers because the rural people have fewer opportunities to receive modern education. They still think that talking about sex is not morally and socially acceptable. Moreover, their intrinsic sociocultural values lead them to deem having sex as a bad behaviour. Thus, they seldom communicate with each other about sex. Due to their relatively lower educational level and poor economic status, they have very few channels to learn knowledge about sex, STDs and AIDS. As the majority of the workers are at a sexually active age and do not live with their wives or girlfriends, they are likely to be involved in commercial sex activities because they live in an environment (urban areas) in which sex is becoming a more publicly acceptable topic. This is consistent with the conclusion reported by Liu et al. (29) and Liu et al. (30). Due to their frequent shifting from one workplace to another, the MMWs do not have a permanent place to settle down. Moreover, due to their relatively lower economic and educational backgrounds, the MMWs are separated from the mainstream local society in which they work and live. In addition, Chinese farmers are likely to maintain close association only with people from the same town or village to establish their own social network, which, in turn, reinforces their isolation from the society. All of these circumstances make them liable to engage in high risk sexual behaviours, more susceptible to STDs and AIDS, (31) and more involved in multi-partner sex and other commercial sex activities. Furthermore, underground commercial sex services are available in many cities (32, 33).

The survey showed that some MMWs had multi-partner, premarital, and extra-marital sex. Multipartner sex is usually connected with commercial sex services, which is consistent with the findings of Wang et al. (3) and Han et al.(34) When former farmers left their hometowns and entered the new environment of their working cities, most of them behaved according to their inborn nature and the surrounding environment of their cities, which is in harmony with the migrant workers' natural desire for sex. Thus, they easily adapt themselves to such an environment. Some of them are involved in sexual activities that expose them to the threat of STDs and AIDS. (35)

It is also found that the usage of condoms among the MMWs was low. Among married migrants, 7.7% and 6.0% used condom with a married partner and with an illegal partner; among unmarried migrants, only 8.6% used condoms during every sexual intercourse. The majority of them did not know how to use condoms appropriately. Among the limited number of those who used condoms, most admitted that their purpose of using condoms was merely for contraception and basically knew nothing about the function of condoms in the prevention of STDs and AIDS. This is consistent with the results of Yan et al. (36) and Liu et al. (29) in China and other Asian countries. (37-38) This suggests that we should introduce more educational interventions and training to increase condom knowledge among MMWs.

In addition, high-risk sexual behaviours, such as alcohol or drug use before sexual intercourse, among some of the MMWs also make them more susceptible to AIDS infection. (39) Commercial sex usually occurs after people consume alcohol. Condom use among drunk people are less frequent compared with people who do not drink or drink little alcohol because alcohol affects judgment and, thereby, promotes risky sexual practices. Studies have shown that intoxicated respondents had weaker intentions to use a condom. (40) Kennedy et al. (41) reported that drug use is one important factor that leads to the infrequent use of condoms. The present survey result is consistent with Kennedy's conclusion. These findings indicate interventions against alcohol use and illicit drug use should be included as an important component in STIs / HIV prevention programs targeted at MMWs.

This survey suggested that the sexual behaviour of MMWs is influenced and restricted by their sexual attitudes. The bivariate logistic analysis also showed that factors affecting married migrant workers' sexual behaviours included their attitudes towards multi-partner, and the factors affecting unmarried migrant workers' sexual behaviours included their attitudes about premarital sex, age, and family income. Thus interventions should address migrant workers' social norms and beliefs surrounding sexual behaviours. Sexual attitudes play an important role in people's relationship with the opposite sex and their behaviours. (42, 43) Zhang et al. (17) believed that, among the factors that influence the spread of STDs and AIDS, sexual conception (which is usually restricted by people's attitudes) plays a significant role. Therefore, in conducting sex education programs among migrant workers, guidance on appropriate sexual attitudes should be provided according to their unique psychological characters and behavioural habits. These findings also indicate a pressing need for targeting sexual attitude education on migrant workers, especially on MMWs who are more tolerant to premarital sex and multi-partner sex, younger unmarried migrant workers, and unmarried MMWs with middle and low family income.

By the latest standard, the poverty threshold in China is 1196 yuan (\$175) per head in 2009. (44) The term "annual income" in this study refers to the total sum of income of a rural migrant worker's family. A rural family in China consists of 3-5 people on the average. Hence, a family with an income lower than 10000 yuan is generally regarded as a low-income family.

Our findings are consistent with some previous studies in Bangladesh, Indonesia, and others Asian countries.(6,7,37,38) For example, in Bangladesh(45), studies have reported high levels of premarital sex (79.4% in married men and 93% in unmarried men), and low condom use in migrant workers, which suggests male migrants have the potential to act as a significant 'bridging population' to the development of a future HIV epidemic. Similarly, in India(46), studies found several factors exposing Nepali migrants to high risk sexual behaviours. These factors included peer norms and pressures, low price sex, lack of family restraint, alcohol use, and low perceived vulnerability to HIV/STI. Previous studies also revealed that many factors were associated with migrant workers' sexual behaviours: age, alcohol and substance use, family structure and socioeconomic status. (26, 45, 46, 47) All of these findings demonstrated migration workers have played a major role in the spread of the HIV/AIDS epidemic not only in China but also in many Asia countries. Moreover, other countries that have abundant MMWs with different cultural and language backgrounds, such as Indonesian/Bangladeshi workers in Malaysia and Singapore, will need involvement of healthcare workers from the same cultural and language backgrounds to better promote sex education in those countries. Thus it is important to have a "holistic" perspective in viewing prevention programs designed to target this population. An intervention program must be responsive not only to individual behaviours, but also to the social forces that drive those behaviours. More importantly, the government should improve the migrants' welfare and social status by abolishing restrictions for migrants and increasing their access to urban facilities such as housing and medical services.

However, the study suffers from several limitations. The data are cross-sectional and the behavioural findings are based on self-reports. Therefore, causal inferences about the findings should be drawn with caution. The survey was done only among migrant workers, without a control group. For example, data on the percentages of condom use among urban counterparts were not compared. China is a multi-ethnical country (56 ethnic groups) with a population of 1.3 billion (90.56%) ethnic Han vs, 9.44% other ethnic groups, such as Mongol, Hui, and Uighur ethnic groups, etc). It is possible that, albeit small, MMWs were likely to be from ethnic minority. It was a pity that our study did not investigate the information on ethnicity of the participants. Homosexual behaviours were not included in the survey. Since participants were recruited in China, our results may not be generalizable to the migrant populations of other Asian countries. We will address these limitations of the study in future and continue to study the sex
attitudes of migrant workers. Intervention efforts should be doubled to address the particular social, cultural, economic and political factors that make MMWs vulnerable to HIV/AIDS.

In conclusion, our study not only examined the characteristics of sexual behaviours in the MMWs but also explored the correlating factors. The results can provide rich data to promote specific interventions for improving their behavioural and sexual health in China. The survey results demonstrate that male migrants have the potential to spread STIs and HIV because of their high-risk sexual practices and low usage of the condoms. This implies not only that a large proportion of migrant men are at high risk of infection, but also that their wives and/or partners are likely to be at risk. Hence, there is a pressing need to devise and launch comprehensive education programs specially designed for MMWs on sex and STIs/HIV.

References

- 1. Weng, N.Q. Social and cultural motivations for transmission of AIDS. Shehuixue Yanjiu 2003; 5: 84-93.
- 2. Wang Y, Zhang YZ, Xu H, et al. Epidemiological Factors for epidemic of AIDS in the countryside in China. Zhongguo Gonggong Weisheng 2003; 19: 1349-50.
- 3. Wang RF, Chen HY, Hu XY, et al. Research on Health Education of Sexually transmitted Disease and AIDS for MMWs from the countryside. Yufang Yixue Qingbao Zazhi 2003; 19: 61-3.
- 4. Lurie MN, Williams BG, Zuma K et al. The impact of migration on HIV-1 transmission in South Africa: a study of migrant and nonmigrant men and their partners. Sex Transm Dis 2003; 30: 149-56.
- 5. Cornman DH, Schmiege SJ, Bryan A et al. An information-motivation-behavioural skills (IMB) modelbased HIV prevention intervention for truck drivers in India. Soc Sci Med 2007; 64: 1572-84.
- 6. Puri M, Cleland J. Sexual behaviour and perceived risk of HIV/AIDS among young migrant factory workers in Nepal. J Adolesc Health 2006; 38: 237-46.
- International Organization for Migration, Joint United Nations Programme on HIV/AIDS (UNAIDS). HIV and Mobile Workers: a Review of Risks and Programmes among Truckers in West Africa. Geneva, Switzerland: International Organization for Migration 2005.
- 8. United Nations Programme on HIV/AIDS (UNAIDS). Population Mobility and AIDS: UNAIDS Technical Update. Geneva, Switzerland: UNAIDS 2001.

- 9. CMOH, UNAIDS, & WHO. Update on the HIV/ STD Epidemic and Response in China 2005. Beijing: China Ministry of Health 2006.
- 10. Wang L et al. The 2007 estimates for people at risk for and living with HIV in China: progress and challenges. J Acquir Immune Defic Syndr 2009; 50: 414-8.
- He, N., Detels, R., Zhu, J., Chen, Z., Fang, Y., Zhang, X., et al. Characteristics and sexually transmitted diseases of male rural migrants in a metropolitan area of Eastern China. Sexually Transmitted Diseases 2005. 32, 286-92.
- 12. Hong Y, Stanton B, Li X et al. Rural-to-urban migrants and the HIV epidemic in China. AIDS Behav 2006; 10: 421-30.
- 13. Yang X, Derlega VJ, Luo H. Migration, behaviour change and HIV/STD risks in China. AIDS Care 2007; 19: 282-8.
- 14. Hesketh T, Li L, Ye X et al. HIV and syphilis in MMWs in eastern China. Sex Transm Infect 2006; 82:11-4.
- 15. Sprecher S, Mckinny K, Walsh R, and Anderson C. A revision of the Reiss premarital sexual of permissiveness scale. Journal of Marriage and the Family 1988; 50:821-8.
- 16. Le Gall A, Mullet E, Riviere S. Age, religious beliefs, and sexual attitudes, The Journal of Sex Research 2002; 39:502-4.
- 17. Zhang WH, Zhao M. Analysis of sexual attitude and STI cognitive level of 337 patients with sexual transmitted disease. Yixue Yu Shehui 2002; 15:53-5.
- Miller P J, Law M, Torzillo P J, Kaldor J. Incident sexually transmitted infections their risk factors in an Aboriginal community in Australia: a population based cohort study. Sex Transm Inf 2001;77: 3745.
- Rou, K., Wu, Z., Sullivan, S. G., Li, F., Guan, J., Xu, C., et al. A five-city trial of a behavioural intervention to reduce sexually transmitted disease/HIV risk among sex workers in China. AIDS 2007;21:S95-S101.
- 20. Wang, B., Hertog, S., Meier, A., Lou, C., & Gao, E. The potential of comprehensive sex education in China: Findings from suburban Shanghai. International Family Planning Perspective 2005;31:63-72.
- Wu, Z., Luo, W., Sullivan, S. G., Rou, K., Lin, P., Liu, W., et al.. Evaluation of a needle social marketing strategy to control HIV among injecting drug users in China. AIDS 2007; 21:S115-S122.
- 22. CMOH, UNAIDS, & WHO. Update on the HIV/STD Epidemic and Response in China 2005. Beijing: China Ministry of Health 2006.
- 23. Yan Hong, Xiaoming Li. HIV/AIDS Behavioural Interventions in China: A Literature Review and Recommendation for Future Research. AIDS Behav 2009; 13:603-13.

- 24. Wang L et al. The 2007 estimates for people at risk for and living with HIV in China: progress and challenges. J Acquir Immune Defic Syndr 2009; 50: 414-8.
- 25. Wang W, Buchhloz ME, Martin MC et al. Prevalence and risks for sexually transmitted infections among a national sample of migrants versus non-migrants in China. Int J STD AIDS 2010; 21: 410-5.
- 26. Liang Z: The age of migration in China. Population and Development Review 2001; 27:499-24.
- 27. China Ministry of Health (CMOH) & World Health Organization (WHO). A Joint Assessment of HIV/ AIDS Prevention, Treatment and Care in China. Beijing: State Council AIDS Committee Office 2007. http://www.chinaids.org.cn/n435777/n443716/appendix/Joint_Assessment_EN.pdf. 15 Fu JF. Sex in China. New York: Plenun Press, 1991; 185-92.
- 28. Liu ZW, Ma X, Xiong WM, et al. Relationship between floating populations from the countryside and transmission of HIV/ADIS. Yufang Yixue Qingbao Zazhi 2002; 18:216-8.
- 29. Liu W, An YD, Shen L, et al. A survey and practice on HIV/AIDS prevention and control among the road construction workers along Yuan-Mo Road in Yunnan Province. Weisheng Ruan Kexue 2002; 16:27-31.
- 30. Xiong LR, Luo HS, Li J, et al. al characteristics and spacial course of floating population and spread of HIV/AIDS. Renkou Yu Jingji 2005; 6:6-10.
- 31. Zhong L, Zhang JC. Investigation into the Health Education programs on sexually transmitted disease and AIDS for floating migrant populations in the labor market.Yufang Yixue Qingbao Zazhi 2003;19:221-2.
- 32. Liu Q, Fu XL, Jia Y, et al. Study of flow education modes on sexually transmitted disease and AIDS among floating populations on the construction sites in Chengdu. Zhongguo Weisheng Shiye Guanli 2003; 6:364-6.
- *33. Han QF, Luo HS, Han JQ. Analysis of sexually deviant behaviour in the process of population flow. Yunnan Shifan Daxue Xuebao 2005; 37:59-64.*
- 34. Zhu GR, Ji CY, Song Y, et al. Qualitative survery into sexual conception and sexual behaviour among floating teenages. Zhongguo Jiankang Jiaoyu 2003; 5: 333-6.
- 35. Yan Z, Li L, Ye XJ, et al. Cross sectional survey of knowledge of and attitude towards AIDS and sex behaviour of MMWs in cities. Zhongguo Nongcun Weisheng Shiye Guanli 2005; 25: 32-4.
- Poudel, K. C., Jimba, M., Okumura, J., Joshi, A. B., & Wakai, S.. Migrants' risky sexual behaviours in India and at home in far western Nepal. Tropical Medicine and International Health 2004; 9:97-103.

- 37. Ravi K. Verma, Niranjan Saggurti, Ajay K. Singh, Suvakanta N. Swain. Alcohol and Sexual Risk Behaviour among Migrant Female Sex Workers and Male Workers in Districts with High In-Migration from Four High HIV Prevalence States in India. AIDS Behav 2010; 14:31-9.
- 38. Conner M, Graham S, Moore B. Alcohol and intentions to use condoms: applying the theory of planned behaviour. Psychology and Health 1999; 14:795-12.
- 39. Wu ZY. Behaviour intervention is currently the effective vaccine for prevention of AIDS. Zhongguo Xingbing Aizibing Fangzhi 2000; 6:221-2.
- 40. Kennedy CA, Skurnick J, Quattrone G, et al. Psychological distress, drug and alcohol use as correlates of condom use in HIV-serodiscordant heterosexual couples. AIDS 1993;7:1493-99.
- 41. Li Z, Shi SH, Ma JD. Current need for carrying out prevention of AIDS and health promotion in migrating populations. Zhongguo Fuyou Baojian 2005; 20:3213-15.
- 42. Sun X, Guo Y, Chang C. Path analysis of influencing factors for safe sex intentions among undergraduates. Zhongguo Weisheng Tongji 2002; 19:261-4.
- 43. http://www.hnkfb.gov.cn/E_ReadNews. asp?NewsID=194
- 44. T. Roy, C. Anderson,, C. Evans,, M.S. Rahman. Sexual risk behaviour of rural-to-urban migrant taxi drivers in Dhaka, Bangladesh: A cross-sectional behavioural survey. Public health 2010; 124: 648-58.
- 45. Smith-Estelle, A., & Gruskin, S.. Vulnerability to HIV/STIs among rural women from migrant communities in Nepal: A health and human rights framework. Reproductive Health Matters 2003; 11: 142–51.
- 46. Shenghui Li, Hong Huang, Yong Cai, Gang Xu, Fengrong Huang and Xiaoming Shen. Characteristics and determinants of sexual behaviour among adolescents of MMWs in Shangai (China). BMC Public Health 2009; 9:195-05.

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Study of bacteriuria in pregnant women and determination of their antibiotic susceptibility patterns

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Abstract

Aim: Urinary Tract Infection (UTI) is one of the most frequently encountered problems owing to significant number of patients needing hospitalization during pregnancy. In pregnant women the incidence of UTI can be as high as 8%. The aims of this study were isolation of pathogenic bacteria from urine culture of pregnant women and determination of their antibiotic sensitivity patterns.

Methods: In this study, midstream urine samples from 139 pregnant women with gestation age ranging between 6 to 38 weeks referred to Obstetrics and Gynecology clinic of Sina hospital, Ahvaz, Iran, were collected. The samples were cultured on Macconkey and Blood agar by calibrated loop method and after overnight incubation at 37°C, standard colony count were performed, which the colony count of 100,000 CFU/ ml or more were considered as serious bacteriuria. The isolates were simultaneously identified using conventional biochemical tests. The antibiotic susceptibility pattern was determined as recommendation of CLSI.

Results: From 139 urine samples, 29(20.9%) were culture positive and colony count of more than 100,000 CFU/ ml. Among them 23 (79.3%) were gram negative and 6 (20.7%) were gram positive bacteria. The most predominant isolate was Escherichia coli 19 (65.5%), followed by Staphylococcus aureus 4 (13.8%), and the lowest rate was belong to Enterobacter with one case (3.4%). Based on the results of microscopic urine examination, 7 (24.1%) of samples revealed the presence of pus cells, and leukocyturia were observed in 11 cases (37.9%). The highest antibiotics sensitivity among gram negative isolates

were seen against ceftazidime, ceftriaxone and cefotaxime, while they showed high resistance to amoxicillin and amoxicillin-clavulonic acid.

Conclusion: Based on overall results, the rate of bacteriuria in examined pregnant women were 20.9% with the common causes of E.coli and Staphylococcus aureus. The most effective antibiotics for most bacterial isolates were cefotaxime, ceftazidime, ciprofloxacin and nitrofurantoin respectively. So it is recommended that routine microbiological analysis and antibiotic sensitivity test of urine samples of pregnant women be carried out before the administration of the drugs for the treatment and management of UTIs to avoid antibiotics resistance.

Key words: Bacteriuria, pregnant women, colony count, antibiotic susceptibility, E. coli.

Introduction

Urinary Tract Infection (UTI) is caused by the presence and growth of microorganisms anywhere in the urinary tract. It is perhaps the single most common bacterial infection of mankind and is an extremely common clinical problem [1].

The urine that stays in the bladder is more likely to allow growth of bacteria and cause infections [2-3]. The importance of UTI is that because it may involve the urethra, bladder, uterus, and kidney [1,2]. The infection affects all age groups, but women are more susceptible than men, because bacteria can reach the bladder more easily in women. This is partially due to the short and wider female urethra and its proximity to anus. Bacteria from the rectum can easily travel up the urethra and cause infections [4, 5]. Approximately 10-15% of women will have a UTI at some time during their life. It is the most common bacterial infections during pregnancy and is one of the most frequently encountered problems owing to significant number of patients needing hospitalization during pregnancy, beginning in week 6 and peaking during weeks 22 to 24. Approximately 90 % of pregnant women develop urethral dilatation, which will remain until delivery (hydronephrosis of pregnancy). [8]. In pregnant women the incidence of UTI can be as high as 8 % [6].

Additionally, the physiological increase in plasma volume during pregnancy decreases urine concentration and up to 70% pregnant women develop glucosurea, which encourages bacterial growth in the urine [7]. UTIs can be asymptomatic, particularly in pregnancy and in the elderly [1, 7]. Women with asymptomatic bacteriuria during pregnancy, are more likely to deliver premature or low-birth-weight infants and have a 20- to 30fold increased risk of developing pyelonephritis during pregnancy compared with women without bacteriuria. The presence of a significant quantity of bacteria in a properly collected urine specimen from a person without symptoms or signs of UTI characterizes as asymptomatic bacteriuria [8]. Several anatomical and hormonal changes in pregnant women lead to ureteral dilatation and urinary stasis, which contribute to the increased risk of developing UTI [9]. Untreated UTIs can lead to complications, such as pyelonephritis, low-birthweight infants, premature delivery, and occasionally, still birth [10]. Therefore, prompt treatment of symptomatic UTI and asymptomatic bacteriuria is required in pregnant women. The importance of coliform bacilli in UTI among pregnant women has long been known in developed countries, and roughly in 80-90% of cases, the most common isolated pathogen is Escherichia coli [3,11-14]. Other responsible microorganisms include other Enterobacteria (Klebsiella, Enterobacter, Proteus), Staphylococcus epidermidis or Staphylococcus saprophyticus, Enterococcus faecalis and group B Streptococcus [10].

The prevalent pathogens of UTIs have been found to be resistant to most chemotherapeutic agents [15, 16]. Antibacterial agents are among the most commonly used medications during pregnancy because treatment of infections is critical to both maternal and fetal well-being [17]. Since screening for and treating asymptomatic bacteriuria is recommended during pregnancy to prevent pyelonephritis and increased maternal and fetal morbidity [18], in present study the prevalence of bacteriuria is investigated in pregnant women.

Methods

This study was performed on 138 pregnant women with gestation age ranging between 6 to 38 weeks referred to Obstetrics and Gynecology clinic of Sina hospital, Ahvaz, south western Iran from 2009 to 2010. Guidelines for proper specimen collection were given to all patients on a printed card [19]. Clean-Catch midstream urine samples were collected in sterile disposable universal bottles (4-5ml) and immediately transferred to the microbiology laboratory for examination or refrigerated at 4°C when the immediate examination was not possible not more than 6 hours after collection. A measured amount of urine, using calibrated loop method was inoculated to nutrient agar medium (Merck, Germany) for colony count. Equal or more than 104 CFU/ml of a single potential pathogen or for each of two potential pathogens interpreted as positive UTI and a result of 102-104 CFU/ml was repeated. A less than102 CFU/ml was interpreted as negative UTI [20].

The urine specimens were cultured on blood and MacConky agar media (Hi-media, Mumbai, India & Merck, Germany) simultaneously for isolation of causative pathogenic organism and incubated at 37 °C for 18-24 hours. All the bacteria isolated from urine were identified using conventional biochemical tests [19]. The urine samples was centrifuged for urinalysis test as well. The smear preparation from the precipitate was examined under the microscope for the presence of yeast cells, parasites, leucocytes, erythrocytes, pus cells and granular casts. For those positive cultures showing the UTI as per colony count results, the antibiotic susceptibility testing of disc diffusion method was performed according to CLSI instructions [21], using common in-use antibiotics for such infections.

Results

From 139 urine samples, 29 (20.9%) were culture positive for UTI with colony count of more than 100,000 CFU/ml, of which 23 (79.3%) were gram negative and 6 (20.7%) were gram positive bacteria. The most predominant bacterium was Escherichia coli 19 (65.5%), followed by Staphylococcus aureus 4 (13.8%), Klebsiella aerogenes 3 (10.3%), Staphylococcus epidermidis 2 (6.9%) and the least common was Enterobacter spp. with 1 case (3.4%) [Table 1]. The patients were divided into 6 age groups, that the highest frequency of isolated bacteria and confirmed UTI were seen



Figure 1. Incidence of UTI in relation to age distributions of pregnant women



Figure 2. The distribution of clinical symptoms among pregnant women with symptomatic UTI

in pregnant women in age group of 21-25 [Figure 1]. Microscopic urine examination was performed for all samples. Seven samples (24.1%) revealed the presence of pus cells, and leukocyturia was observed in 11 samples (37.9%). The burning during urination was the most common complain in 12 patients (41.3%). Other symptoms were as lower abdominal pain in 9 cases (31%), Urine retention 8 (27.6%), flank pain 5 (17.2%), and hematuria 10 (34.5%) [Figure 2]. Asymptomatic infections were discovered in 7 patients (24.1%). Among total 29 positive cases, 18 samples were taken from hospitalized pregnant women and 11 samples from outpatients. The highest rate of UTI was seen in those patients with gestational age of 10 -19 weeks (37.93%) and more than 30 weeks (31%) [Table 2]. According to results from antibiotics susceptibility testing, the highest resistance



Figure 3. Antibiotic resistance pattern among isolated bacteria from UTIs of pregnant women

Table 1.	The	frequency	of isol	lated b	acteria	from	UTI	of hos	pitalized	and or	itpatient	pregnant	women
			./					./				,	

Isolated hostoria	$N_{0}(0/)$	E	Iospitalized	Outpatients		
Isolated Dacteria	110.(70)	≤10	50000-100000	≤10	50000-100000	
Escherichia coli	19(65.6%)	13	3	3	0	
Klebsiella	3(10.3%)	2	0	1	0	
Enterobacter spp.	1(3.4%)	0	0	1	0	
Staphylococcus aureus	4(13.8%)	0	0	3	1	
Staphylococcus epidermidis	2(6.9%)	0	0	0	2	
Total	29(100)	15	3	8	3	

Table 2. The frequency of UTI in relation to gestational age grouping in present study

Positive culture No. (%)	Patients No (%)	Gestation age
6 (20.68)	18 (12.94)	Less than 10 weeks
11(37.93)	34 (24.46)	10-19 weeks
3(10.34)	52 (37.41)	20-29 weeks
9 (31)	35 (25.18)	More than 30
29	139	Total

were seen against amoxicillin and amoxicillin-clavulanic acid, while the isolates showed the lowest resistance to ceftazidime, ceftriaxone and cefotaxime [Figure 3].

Discussion

UTIs are one of the most common bacterial infections during pregnancy which are associated with risks to both the fetus and the mother, including pyelonephritis, preterm birth, low birth weight, and increased perinatal mortality [16]. Remarkable changes occur in the structure and function of the urinary tract during pregnancy that totally results in urinary stasis the condition that along with the presence of vesico-ureteral reflux predispose some women to upper UTIs and acute pyelonephritis. Also hormonal change of progesteron and estrogen may lead to a decreased ability of the lower urinary tract to resist invading bacteria. Moreover, up to 70 % of pregnant women develop glycosuria, which encourages bacterial growth in the urine [2].

In this study the prevalence of UTI among tested pregnant women was 20.9%. This finding was in agreement to the study of Ah-Haddad who reported a prevalence of 30% in a similar work [22]. However our UTI rate was lower than the incidence of 58% reported by Onifade et al. [23], 47.5% reported by Okonko et al. [15] and 45% reported by Subedi et al. [24]. The differences between the incidence rate of UTI in these studies could be due to differences in the environment, social habits of the community, the standard of personal hygiene and differences in education as stated elsewhere[22]. In our study E. coli (65.5%) and S. aureus (13.8%) were the most common bacteria isolated from the tested samples. This was in agreement with similar studies isolated E. coli and S. aureus from UTI in pregnant women as the most prevalent bacteria [15, 22], and studies reported E. coli as the most common organism in UTI [24-26]. Asymptomatic bacteriuria was noticed in 24.9% of pregnant women in this study. The rate of asymptomatic UTI reported vary in different similar studies. Hamdan et al. [27] and Obirikorang et al. [28], reported rates of 14.7% and 9.5% bacteriuria among the tested pregnant women respectively. The lower incidence of UTI was reported in the study of Jazayeri Moghadas and Irajian as 3.3% [25]. This rate is belonged to the cases of asymptomatic UTI that are normally underestimated in the certain populations of community due to lack of a regular screening program.

Based on the results from antibiotics susceptibility testing, the resistance of the isolates to used antibiotics were high in this study. The American College of Obstetricians and Gynecology stated that antibiotic resistance in uropathogens is increasing worldwide. It varies according to geographic locates and is directly proportional to the use and misuse of antibiotics. Understanding the impact of drug resistance is of the critical importance as the changing rate of antibiotic resistance has a large influence on the empirical therapy of UTIs [29].

In present study E. coli as the most prevalent isolate, showed the high sensitivity to ceftazidime which was in agreement with other works presented the similar findings[22, 25]. The isolated gram negative bacteria in this study showed the high to moderate sensitivities to nitrofurantoin (70%) and nalidixic acid (54.2%). This rate of sensitivity was lower compared to what previously reported by Okonko et al. [15]. In their study E. coli as the most common isolate, showed full sensitivity to nitrofurantoin and 75% sensitivity to nalidixic acid [15]. In conclusion, the findings of this study revealed that the prevalence of bacteriuria was 20.9% among tested pregnant women with important infecting organisms were found to be E. coli and S. aureus. This study has highlighted the need to raise awareness of UTIs and to expand services for prevention and treatment for pregnant women. We found 24.1% asymptomatic bacteriuria among our tested patients, so it is therefore recommended that routine microbiological analysis and antibiotic sensitivity test of mid stream urine samples of pregnant women be carried out before the administration of the drugs for the treatment and management of UTIs since resistance to these drugs are developing in the community.

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References

- Ebie MY, Kandakai-Olukemi YT, Ayanbadejo J, Tanyigna KB. Urinary tract infections in a Nigerian Military Hospital. Niger J Microbiol 2001; 15(1): 31-37.
- Alemu A, Moges F, Shiferaw Y, Tafess K, Kassu A, Anagaw B, Agegn A. Bacterial profile and drug susceptibility pattern of urinary tract infection in pregnant women at University of Gondar Teaching Hospital, Northwest Ethiopia. BMC Res Notes. 2012; 5(1):197.
- 3. National Kidney and Urologic Diseases Information Clearinghouse (NKUDIC). Fact sheet: Urinary tract infections in adults. NIH Publication No. 06-2097, 2005.
- 4. Awaness AM, Al-Saadi MG, Aadoas SA. Antibiotics resistance in recurrent urinary tract infection. Kufa Medical Journal 2000; 3:159.
- Kolawole AS, Kolawole OM, Kandaki-Olukemi YT, Babatunde SK, Durowade KA, Kolawole CF. Prevalence of urinary tract infections (UTI) among patients attending Dalhatu Araf specialist hospital, Lafia, Nasarawa State, Nigeria. Int J Medicinal Med Sci 2009; 1(5):163-167.
- 6. Delzell JE, Lefevre ML. Urinary tract infections during pregnancy. Am Fam Physician 2000; 61(3):713-721.
- 7. Hill JB, Sheffield JS, McIntire DD, Wendel GD Jr: Acute pyelonephritis in pregnancy. Obstet Gynecol 2005;105(1):18-23.
- 8. Schnarr J, Smaill F. Asymptomatic bacteriuria and symptomatic urinary tract infections in pregnancy. Eur J Clin Invest. 2008; 38 Suppl 2:50-57.
- Dwyer PL, O'Reilly M. Recurrent urinary tract infection in the female. Curr Opin Obstet, Gynecol 2002; 14(5):537-543.
- 10. Macejko AM, Schaeffer AJ. Asymptomatic bacteriuria and symptomatic urinary tract infections during pregnancy. Urol Clin North Am 2007; 34:35-42.
- 11. Le J, Briggs GG, McKeown A, Bustillo G. Urinary tract infections during pregnancy. Ann Pharmacother 2004; 38(10):1692-701.
- 12. Christensen B. Which antibiotics are appropriate for treating bacteriuria in pregnancy? J Antimicrob Chemother 2000; 46(Suppl 1):29-34.
- 13. Gilstrap LC, Ramin SM. Urinary tract infections during pregnancy. Obstet Gynecol 2001; 28:581-591.

- 14. Ovalle A, Levancini M. Urinary tract infection in pregnancy. Curr Opin Urol 2001; 11(1):55-59.
- Okonko IO, Ijandipe LA, Ilusanya OA, Donbraye-Emmanuel OB, Ejembi J, Udeze AO, Egun OC, Fowotade A, Nkang AO. Incidence of urinary tract infection (UTI) among pregnant women in Ibadan, South-Western Nigeria. African J Biotech 2009; 8 (23):6649-6657.
- Taneja N, Rao P, Arora J, Dogra A. Occurrence of ESBL & Amp-C beta-lactamases & susceptibility to newer antimicrobial agents in complicated UTI. Indian J Med Res 2008;127(1):85-88.
- 17. Nicolle LE. Asymptomatic Bacteriuria: When to screen and when to treat. Infect Dis Clin North Am 2003; 17 (2): 367-394.
- Crider KS, Cleves MA, Reefhuis J, Berry RJ, Hobbs CA, Hu DJ. Antibacterial medication use during pregnancy and risk of birth defects: National Birth Defects Prevention Study. Arch Pediatr Adolesc Med 2009; 163:978-985.
- Forbes BA, Sahm DF Diseases. 7th Edn., Amazon: Philadelphia, Vol.2, 2010. Pp.881-882., Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 12th Edn., The Mosby Company Inc., St. Louis, 2007 pp. 109-214, 842-855.
- 20. Mandell GL, Bennett JE Dolin R. Mandell, Douglas, and Bennett's Principles and practice of infectious diseases. 7 Edn., Amazon: Philadelphia, Vol. 1, 2010. Pp.881-882.
- 21. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 12th Informational Supplement, CLSI Document M100-S12, Pennsylvania, USA. 2002.
- 22. Al-Haddad AM. Urinary tract infection among pregnant women in Al-Mukalla district, Yemen. Eastern Mediterranean Health Journal 2005; 11(3): 505-510.
- 23. Onifade AK, Omoya FO, Adegunloye DV. Incidence and control of urinary tract infections among pregnant women attending antennal clinics in government hospitals in Ondo State, Nigeria. J Food Agric Environ 2005; 3(1): 37-38.
- 24. Subedi M, Basnyat SR, Acharya SD. Urinary tract infection in pregnancy and its correlation with nitrite test. J Nepal Health Res Counc 2009; 7(15):80-83.
- 25. Jazayeri Moghadas A, Irajian G. Asymptomatic urinary tract infection in pregnant women. Iranian J Pathol 2009; 4 (3):105-108.

- 26. Krcmery S, Hromec J, Demesova D. Treatment of lower urinary tract infection in pregnancy. Intern J Antimicrob Agents 2001; 17:279–282.
- 27. Hamdan HZ, Ziad ABM, Ali SK, Adam I. Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. Annals Clin Microbiol Antimicrob 2011; 10:2-6.
- 28. Obirikorang C, Quaye L, Bio FY, Amidu N, Acheampong I, Addo K. Asymptomatic bacteriuria among pregnant women attending an-tenatal clinic at the University Hospital, Kumasi, Ghana. J Med Biomed Sci 2012; 1(1): 38-44.
- 29. American Academy of Pediatrics and American College of Obstetricians and Gynecology. Guidelines for Perinatal Care. American Academy of Pediatrics. 6th Edn. 2007.

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Surgical treatment of paranasal sinus mucoceles: Our experiments and literature review

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Abstract

Mucoceles are epithelial bordered, mucus containing structures that completely fill the paranasal sinuses. They are most commonly seen in frontal sinus and then ethmoid, maxillary and sphenoid sinuses. As they grow bone erosion may be caused due to environmental pressure and they can cause some different symptoms by overflowing outside the sinus. Untreated mucoceles may result in serious morbidity according to their placements. Aggressive mucoceles can erode the sinus wall. In the case of frontoethmoid mucoceles extending to the orbita, patients can commonly apply with the complaints of diplopia, headache and outer pushing of the eye. In treatment; surgical procedures like open surgery or most recently recommended endoscopic endonasal marsupialisation are performed according to the state of the patient. In this article we discussed the patients applied to our clinic with mucocele with review of the literature.

Keywords: Mucocele, paranasal sinus, frontoethmoid mucocele, endoscopic sinus surgery, external approach.

Introduction

Mucoceles are the epithelial restricted structures which contain mucus and completely fill inside the paranasal sinus (1). Mucoceles are typically seen after the fourth decade of life regardless of a sex discrimination. They are most frequently observed in the frontal sinus and ethmoid, maxillary and sphenoid sinus follow this, respectively (2). The most encountered etiological reasons of mucocel are previous nasal surgery, chronic sinusitis, nasal polyposis and nasal trauma (3-4). Symptoms and signs of mucocele depend on the lesion's location and the size of the lesion detriment the bone (5). The lesion obliterating the frontal sinus may cause erosion of the anterior and posterior bone walls. Lesion can spread to the orbita and intracranial structures with this way (6-7). Periorbital pain, ptosis, frontal headache with proptosis may exist at the frontal sinus mucoceles and diplopia may occur due to the repression of the eyeball against down to the outside (8). Damage of the sinus posterior wall by mucocele can lead to menengitis, meningoencephalitis, pneumocephalus, brain abscess, seizures and fistula of the cerebrospinal fluid. Cranial nerve paralyzes may occur in rare cases (9). For this reason untreated mucoceles are the causes of mortality and morbidity according to their locations. Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are important diagnostic devices for diagnosis and planinng of the surgery.Paranasal sinus carcinoma, aspergillus infection and chronic infections should be considered for differantial diagnosis (9).Surgical treatment includes functional endoscopic sinus surgery, craniotomy with or without sinus obliteration and craniofacial approaches. More aggresive interventions are required in situations like infiltrations of orbita and middle fossa (10). Ethmoid and sphenoid sinus mucoceles are often treated with marsupialization, frontal sinus mucoceles are frequently treated with both marsupialization and radical interventions (11).

Materials and methods

With co-working of Sivas Cumhuriyet University Faculty of Medicine, ENT Department and Elazığ Training and Research Hospital ENT Clinic,10 cases were included to the study who underwent surgery with paranasal sinus muco-

cele diagnosed between the dates of 2005-2012. Cumhuriyet University Faculty of Medicine Ethics Committee approval was taken for the study. Mucoceles had caused expansion at sinus walls in all cases. Three of these patients have had frontal, four have maxillary and the rest have ethmoidal mucoceles. Two of the frontal mucoceles, and one of the maxillary mucoceles so as to three cases were treated with combined approach (functional endoscopic sinus surgery plus external approach or functional endoscopic sinus surgery plus cald Well Luc technique), and seven patients remaining were treated with functional endoscopic sinus surgery alone.

Statistical method

Data were analyzed in SPSS computer program (ver.14.0), descriptive statistics of the individuals who were enrolled to the study were expressed as numbers and percentages and p<0.05 was considered significant.

Results

Seven of the patients were male (70%), and three were female (30%). The mean age was 33.5 (min. 7- max.60). Four (40%) of these individuals had maxillary sinus mucocele, three (30%) had frontal sinus mucocele and the other three patients (30%) had ethmoid sinus mucocele. Seven of the patients were treated with endoscopic procedure and the rest three ones were treated with combined surgical procedure (endoscopic+external approach). Median follow up duration was 15 months (3-84). Location of the mucoceles and surgical approaches were shown in the Table 1.

No recurrence was observed in other patients except one of the cases who have had ethmoidal mucocele. Mucocele was showing expansion towards frontal and orbital region in this case of recurrence. This case who developed recurrence for four times has been attempting to treated with endoscopy each time.

Discussion

Mucoceles may present with different symptoms according to the localization. These symptoms may be ophthalmological, rhinological and neurological. The most common ophthalmological symptoms are; palpebral edema and proptosis. While most frequent rhinological symptoms are rhinitis and nasal obstruction, on the other hand, headache is the most frequent neurological symptom (3). Mucoceles originated from the frontal and ethmoidal sinuses are clinically silent and may spread to the orbita and anterior cranial fossa during the disease process. Necrosis due to the compression and releasing of the natural osteolytic factors cause destruction of the bone structures (12). Morbidity and potantial mortality may occur depending upon neighborhood to the orbita and brain and disease progresses to surrounding structures. Greatest majority of the frontal mucosal cases apply to the hospital at late period when the orbital or cranial invasion occured already. For this reason, the most common symptoms are headache and visual defects (12).

Mucocele has been defined for more than a century but etiology is not completely known. Occlusion of the frontal recess (13) or loss of drainage property of sinusal mucosa (10) were implicated in the etiology. These patients often have a history of trauma and this situation supports that trauma can be an etiological factor. Not to excising of frontal sinus mucosa exactly during cranialization was reported as a reason for mucocele formation (14). Although mucoceles are benign lesions, they

Table 1. Location of paranasal sinus mucoceles and the surgical procedures performed

		Surgical P	Total		
		Endoscopic	Combined	Iotai	
	Maxillary	3 %75	1 %25	4 %100	
Location	Frontal	1 %33.3	2 % 66.7	3 %100	
	Ethmoid	3 %100	0 % .0	3 %100	
Total		7 %66.7	3 %33.3	10 %100.0	

As seen in table 1 two of three frontal sinus mucocele (66,7%) are treated with combined procedure. In both of theses cases mucoceles were widespread. There was extension to dura and orbital roof. Only one of the mucoceles in maxillary sinus was treated with combined procedure. Endoscopic sinus surgery was sufficient in other cases.

require surgical treatment for potential mass and osteolytic effects. However, there is not an accurately accepted method for surgical approach. An ideal surgical approach should be able to solve the problem completely as preventing recurrence without any distruption of paranasal sinuses and natural drainage ways and will not be contradictory to the nasal physiology (3). The election of the surgical procedure must be done due to the localization, size and spreading out the environment structures of the mucocele. Surgical treatment of the frontal mucoceles includes the methods like endoscopic drainage, drainage with transcaruncular approach, external fronto-ethmoidectomy and sinus obliteration application with osteoplastic flap technique till today (1, 15, 16). Endoscopic approach is a more effective and faster method and has less morbidity. It less damage nasal structure and physiology than other techniques. Other important advantage is the patients return to normal life sooner without any external scar.

Serrano et al. (3) have treated 33 of the 60 cases (66,6%) with frontal and frontoethmoidal mucoceles with endoscopic approach, 27 cases with external approach combined with endoscopic approach or only with external approach. Complications as palpebral edema, local infections and headache were observed in two of the patients they treated with endoscopy, and 6 of the other patients. They established that there was not any stenosis in cases treated with marsupialization during their 36 months follow up. Kennedy et al.(17) expressed that any recurrence did not develop in 9 of the 11 patients with frontal mucoceles who were performed endoscopic marsupialization at their 17.4 months follow up. Similarly Har-El et al. (18) marsupialized 112 patients with endoscopy and reported that there was not any recurrence during 32 months follow up. But, endoscopic approach is not sufficient for all fronto-ethmoid mucoceles. For lateral localized mucoceles or in cases with mucoceles which extending beyond the sinus or spreading inside the orbita and also in cases with an excessive scar in frontal recess due to the trauma or previous surgery, combined approach or further open techniques are necessary (2,19).

In the mucoceles opening to adjacent anatomical structures, bacterial and neurological complications are seen extensively (10).

It is a controversial topic that the removing of the wall of mucocele during the operation. The most reliable way on the treatment of mucocele is entirely removing of the sinus mucosa. Although the removing of cyst wall have been suggested by some authors, it has been advocated the view that the mucocele wall displays active mucociliary transport and acquires normal mucosa after a time, therefore protection of cyst wall has been also defended. In the cases of fronto-orbital mucocele, the disadvantages of radical interventions are to be high of morbidity and cosmetic defect due to scar tissue. In the cases performed obliteration, the field of operation can not be evaluated radiologically. Chiarini et al. claimed that endoscopic sinus surgery in non-invazive frontal mucoceles, cranialization and nasofrontal duct obliteration in cases with eroded frontal sinus posterior wall and infiltrating dura are the most convenient approaches (10). Delfini et al. have performed the adaptation of surgical options according to the cases in the fronto-orbital mucocele patients. In these patients, Weitzel et al. have suggested the providing of wide fronto-ethmoid exposition with osteoplastic flap, fully cleaning of orbital and intracranial components of the mucocele, and the making of calvarial bone reconstruction of orbita and anterior cranial fossa.

Conclusion

We have thought that in the cases with frontoorbital mucocele progressed to adjacent structures via bone destruction, but have not osteitis and fistula, while the result could be arrived with the help of endoscope and the minimal invasive surgery to more destructive operations; in the frontal sinus mucoceles that have osteitis and fistula or led to erosion on the dura and orbita roof, and in the frontoethmoid mucoceles that have orbital invasion, functional endoscopic sinus surgery should be combined with external approach..

References

1. Hulett KJ, Stankiewicz JA. Primer sinüs cerrahisi. Cummings Otolaringoloji Baş ve Boyun Cerrahisi. Çeviri editörü Koç C. Güneş Tıp Kitapevi, Ankara. 2007; 1229-54.

- 2. Busaba NY, Salman SD. Ethmoid mucocele as a late complication of endoscopic ethmoidectomy. Otolaryngol Head and Neck Surgery. 2003; 128: 517-22.
- 3. Serrano E, Klossek MJ, Percodani J, Yardeni E, Dufour X. Surgical management of paranasal sinus mucoceles: A long term study of 60 cases. Otolaryngology Head and Neck Surgery. 2004; 131:133-40.
- Özcan M, Akdoğan Ö, Gün T. Giant mucocele of the maxillary antrum. Türk Otolarengoloji Arşivi. 2002; 40(2):150-2.
- Jayaraj SM, Patel SK, Ghufoor K, Frosh AC: Mucoceles of the maxillary sinus. Int J Clin Pract. 1999; 53: 391-3.
- 6. Chiarini L, Nocini PF, Bedogni A, Consolo U, Giannetti L, Merli GA: Intracranial spread of giant frontal mucocele: A case report, Br J Oral Maxillofacial Surg. 2000; 38:637-40.
- Lai PC, Liao SL, Hou PK: Transcaruncular approach for the management of frontoethmoid mucoceles. Br J Ophtalmol. 2003; 87:699-703.
- 8. Raman M, Peter W, Dinesh S: Bilateral dynamic proptosis due to frontoetmoidal sinus mucocele. Ophtal Plast Reconstr Surg. 2003; 19:156-7.
- 9. Cagigal BP, Lezcano JB, Blanco RF, Cantera JMG, Cuéllar LAS, Hernández AV: Frontal sinus mucocele with intrcranial and intraorbital extension. Med Oral Patol Oral Cir Bucal. 2006; 11:527-30.
- 10. Chiarini L, Nocini PF, Bedogni A, Consolo U, Giannet- ti L, Merli GA. Intracranial spread of giant frontal mucocele: a case report, Br J Oral Maxillofacial Surg. 2000; 38:637-40.
- 11. Uzun KH, İleri F, Akman E, Erkam Ü. Frontoetmoid mukosellerde endsokopik yaklasım, KBB ve Bas Boyun Cerrahisi Dergisi. 1996; 4:39-43.
- 12. Weitzel EL, Hollier LH, Calzada G, Manolidis S. Single stage management of complex fronto-orbital mucoceles, J Craniofac Surg. 2002; 13:739-44.
- 13. Atasoy Ç, Üstüner E, Erden İ, Akyar S. Frontal sinus mucocele: A rare complication of craniofacial dysplasia, J Clin Imaging. 2001; 25:388-91.
- 14. Raman M, Peter W, Dinesh S. Bilateral dynamic proptosis due to frontoetmoidal sinus mucocele. Ophtal Plast Reconstr Surg. 2003; 19:156-57.
- 15. Kelly A, Malloy OD. Fronto-ethmoid sinüs mucocele.Journal of the American Optometric Association. 2006; 77(9): 450-8.

- Lai PC, Liao SL, Jou JR. Transcaruncular approach for the management of frontoethmoid mucoceles. British Journal of Ophthalmology. 2003;87:699-703.
- 17. Kennedy DW, Josephson JS, Zinreich J. Endoscopic sinüs surgery for mucoceles: a viable alternative. Laryngoscop. 1989; 99:885-95.
- Har-El G, Balwaly AN, Lucente FE. Sinus mucoceles: is marsupialization enough? Otolaryngol Head Neck Surg. 1997; 117:633-40.
- 19. Herndorn M, McMains CK, Kountakis ES. Presentation and management of extensive frontoorbital ethmoid mucoceles. American Journal of Otolaryngology–Head and Neck Medicine and Surgery. 2007; 28:145-7.
- 20. Lai PC, Liao SL, Hou PK. Transcaruncular approach for the management of frontoethmoid mucoceles, Br J Ophtalmol. 2003; 87:699-703.
- Kennedy DW, Josephson JS, Zinreich SJ, Mattox DE, Goldsmith MM. Endoscopic sinus surgery for mucoceles: a viable alternative. Laryngoscope. 1989 Sep;99(9):885-95.
- 22. Delfini R, Missori P, Ianetti G, Ciapetta P, Cantore G. Mococeles of the paranasal sinuses with intracranial and intraorbital extension: report of 28 cases. Neurosurgery. 1993; 32:901-6.

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The change and differences in exercise types in association with dementia proteins in Korean elderly females

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Abstract

The purpose of this study is to prove the association with dementia proteins (β-amyloid, DHE-As) of Korean elderly females by different types of an exercise. The subjects of this study were total 27 of Korean elderly females aged between 59 years and 69 years among members of B fitness center in Gyounggi-Do. We classified them into three groups with 9 subjects each. AGE(Aerobic Exercise Group), REG (Resistance Exercise Group), and SEG (Sensory Integration Exercise Group) were assessed in August 2011. The result was recorded after sensory integration exercise of 60 minutes in 3 times per a week during 12 weeks. The before and after density transition of β -amyloid of within groups by types of exercise was positively reduced and AGE and REG group showed significant difference (p < .05) and SEG group also showed significant difference (p < .01). However, group by types of exercise did not show significant in AEG, REG and SEG.

And then before and after density transition of DHEAs within group by types of exercise showed significant difference (p<.001) in AEG, REG and SEG. And the before and after density transition of DHEAs within group by types of exercise showed significant difference (p<.05) in REG and SEG, and showed higher values in SEG than REG.

In the result of this study, the density transition of β -amyloid was reduced by the types of each exercise and DHEAs was increased. Therefore, Aerobics Exercise, Resistance Exercise and Sensory integration are judged as exercise way to reduce the risk factor of dementia. Regular and continuous exercise is effective way to prevent dementia in old females group.

Key words: β-amyloid, DHEAs, dementia.

Introduction

Aging is a term used to explain biological, psychological, physiological changes when there is reduction of ability to resist stress, damage and diseases in the cells, tissues and organs due to maturity of human bodies (1). Currently, South Korea has become an aging society because in 2005 the population of those over 65 of age exceeded 9.1% of the total population. In 2010, the elderly population would have reached 10.9% and in 2018 it will reach 14.3%, South Korea will become one of aged societies and in 2026 the aged population will reach 20.7% making it a super-aged society. The increase in the medical expenses of this population became a social issue (2).

According to the National Bureau of Statistics South Korea (2), among elderly people between the age of 65 and 69 in 2010, the number of elderly with dementia would have reached 38,000. The onset rate is gradually increasing. The bureau estimates that as the elderly population grows, until 2020 around 8.4% of the population of those over 65, over 620,000 would have senile dementia.Dementia is the most frequently appearing cause of most of the psychological symptoms for old age. It chronically and gradually progresses and it is a disability in cognitive functions and perceptions which includes damage in memory, reason, learning ability and judgment (3-4). There are various kinds of dementia, but Alzheimer's disease is the most common of all which is around 50-60% of the total population with dementia. The second common type would be vascular dementia which makes up of 20-30% and others which make up around 10-30%. 50-60% of those with dementia and over 65 of age suffer from Alzheimer and female elderly are reported to show a higher level of Alzheimer type dementia than male counterpart (5).

There are three types of genes which are known to cause Alzheimer and among them APP gene is located in the 21st chromosome and encodes amyloid precursor protein. The mutation and hyper-expression of this type of gene will increase production of β -amyloid, cellulotoxic substance (6). Also β -amyloid consists of 38 to 43 types of amino acid and it is reported that if β -amyloid is over produced and if they accumulate in the brain cells then dementia will develop (7).

Together with β -amyloid, another factor which has close relationship with dementia would be Dehydroepiandrosterone sulfate(DHEAs). This factor is a type of steroid hormone which can be found in the blood at a high level of concentration and can be over produced in the adrenal cortex. The level of DHEA reduces as they grow older and became biological indicator for cancer, diabetes, Alzheimers and hypomnesia (8). It is reported that those with low concentration level of β-amyloid and higher concentration level of DHEAs would have a higher chance of developing dementia than those without (8). Many previous studies report that various forms of exercise show positive effect on dementia, but they are mostly focusing on aerobic exercises and resistance exercises and other limited forms of exercises and the subjects of many of these studies are animals.

However, dementia would cause serious disability in carrying out routine activities, as more than two factors of many cognitive abilities would fail due to dementia without losing consciousness. To name a few, memory failure, loss of speech, failure of recognition of time and space and loss of ability among others. Therefore not only aerobic exercises and resistance exercises but also other exercises would be necessary to give impact on the sensory organs. Accordingly, the purpose of this study is to find out the effect of aerobic exercises, resistance exercises and various forms of sensory-impact exercises on the concentration level of β -amyloid and DHEAs, all of which are related to dementia.

Methods

Subject

This experiment was conducted with total of 27 Korean elderly females at their age between

59-69 who were the members of B fitness center at Gyeonggi-Do, Korea. They were divided into three different groups; AEG(aerobics exercises group) with 9 subjects, REG(resistance exercises group) with 9 subjects and SEG(various forms of sensory-impact exercises group) with 9 subjects. They heard overall information on the study and they signed the consent forms. They also went through medical examination done by specialist doctors. The subjects' physiological characters are shown in table 1. All study procedures were approved by the Institutional Review Board at Dan Kook University in South Korea.

Experimental procedures

In order to analyse the changes in β -amyloid and DHEAs of the elderly females at their age between 59-69 according to their exercise types, the subjects's blood samples were taken and they all did the pre-tests before the actual experiments.

The exercise program was structured to include aerobic exercise, resistance exercise and sensoryimpulse exercise for the duration of 12 weeks. After the experiment, the subjects' blood samples were taken and the changes in their β -amyloid and DHEAs were analysed.

Exercise program

The exercise program for this study consists of aerobic exercises, resistance exercises and sensory-impact exercises for the elderly female subjects for the duration of 12 weeks. Also the cardiovascular endurance was set for %HRR 40~60% which was recommended by ACSM (9) in 2001. During each set of the exercise program, an adequate level of rest and water intake and consistent level of nutrition intake would be recommended.

For the aerobic exercise, warm-up and cool-down took 10 minutes and the main exercise took 40 minutes on treadmill. Resistance exercise was done with 10 minutes of warm-ups and cool-downs. The main exercise was done at the intensity of 60~70% per 1RM of increase based on the muscular strength. Three sets of 12 counts were done for the duration of 40 minutes. Sensory impact exercise was done with 10 minutes of warm-ups and cool-downs. For the main exercise, another exercise program with Q-Trainer was modified to fit the purpose of this study and the duration was 40 minutes. Sensory impact exercise program was developed for the purposes of improving timing, rhythm, concentration and motion planning based on the brain principle.

This program was based on improvement on neurons also known as neuroplasticity and neuro-remodelling and it integrated neurosensory and neuromotor program. It would stimulate multiple senses, integrating auditory stimulus and motion stimulus through the training in the areas of synchronization, timing and rhythmicity. This program consists of mat exercises, trampoline, balance board, Gym ball, walking on the balance beam, throwing and catching a ball and hopping on one leg among others. The intensity of the exercise program was measured using one of the indirect measuring method, namely Karvonen method(10). For the aerobic exercise, the intensity was measured using wireless heartbeat monitor of the treadmill. The intensity of resistance exercise and the sensory integrated exercise was set for %HRR 40~60% and was measured in every 10 minutes.

Method of drawing blood and blood analysis

The subjects went without food for 12 hours before the blood test. They took an adequate level of rest before the blood was drawn and the blood was taken from the antecubital vein of their upper arms using disposable syringes. In order to analyze the concentration level of β -amyloid and

Table 1. Characteristics of the subjects (Mean \pm SD)

DHEAs in the blood, 10ml of venous blood was taken from each subject. After the venous blood was taken in the evacuated blood collection tube without anticoagulants for the purposes of serum separation the blood was left both at the room temperature and at 4°C for one hour. After one hour the blood was put in the centrifugal filter and was centrifuged at 3000rpm for 15 minutes and was separated into serum and plasma. The blood was stored at -80°C until the analysis. The analysis was conducted by N company, adopting standardized ELISA method.

Statistical analysis

For the process of the collected raw data, SPSS18.0(SPSS, Chicago, IL, USA) for Window was used to estimate the average and standard deviation for each variant, In order to analyze and estimate the changes in manifestation of β-amyloid gene and, DHEAs before and after the application of the exercise program, paired t-test was adopted. In order to analyze the difference among the groups, one-way ANOVA was undertaken. Scheffe was adopted for Posteriori test and the level of statistical difference was p < .05.

Results

The purpose of this study is to explore the impact of the difference in the type of the exercise on β-amyloid and DHEAs of Korean elderly females.

	(n)	age (year)	height (cm)	weight (kg)	%fat (%)
AEG	9	66.3±4.2	155.2±5.5	54.5±6.2	28.4±5.2
REG	9	67.1±3.3	156.1±6.3	53.6±5.8	27.2±3.8
SEG	9	66.9±4.3	154.3±4.4	54.7±5.7	28.8±4.5

AEG: aerobics exercises group, REG: resistance exercises group SEG: various forms of sensory-impact exercises group

Table 2. The changes in the β -amyloid of the Korean old female subjects before and after the performance of different types of the exercise program

		Means±SD	t	Sig	
	pre	1.4933±1.01816	2 5 9 0	022*	
AEO(N-9)	post	.8211±.64702	2.380	.033*	
$\mathbf{DEC}(\mathbf{N}=0)$	pre	1.5144±1.93650	2 2 4 9	.045*	
$\operatorname{KEO}(N-9)$	post	.9578±1.26898	2.348		
SEC(NI=0)	pre	1.8467±.91423	5 505	001**	
SEG(N-9)	post	.6267±.36562	5.303	.001**	

*p<.05, **p<.01, ***p<.001

1) The changes in the β-amyloid of the Korean elderly females subjects before and after the performance of different types of the exercise program. The concentration level of β-amyloid in all of AEG exercise group, REG exercise group and SEG exercise group reduced from 1.4933 ± 1.01816 to $.8211\pm.64702$, from 1.5144 ± 1.93650 to $.9578\pm1.26898$, and from $1.8467\pm.91423$ to $.6267\pm.36562$ respectively. They all showed statistically significant difference (p<.05)(p<.05)(p<.01) respectively.

2) The changes in the DHEAs of the Korean elderly females subjects before and after the performance of different types of the exercise program. The concentration level of DHEAs of all of AEG exercise group, REG exercise group and SEG exercise group increased from 40.0111 ± 8.94964 to 74.5222 ± 19.71996 , from 47.8000 ± 23.64017 to 86.5444 ± 36.32823 and from 53.2667 ± 30.78559 to

 100.2889 ± 40.74290 respectively. They all showed statistically significant difference (p<.001)(p<.001) (p<.001) respectively.

3) Changes in β -amyloid of the Korean elderly females compared among groups performing different types of exercise. The result of variance analysis of Changes in β -amyloid did not show statistically significant difference.

4) Changes in DHEAs of the Korean elderly females compared among groups performing different types of exercise. The result of variance analysis of Changes in DHEAs showed statistically significant difference. In order to identify changes in DHEAs of the elderly female compared among groups performing different types of exercise, posteriori test was performed using scheffe multiple comparison method and the result shows higher level in SEG compared to the REG(p<.05).

Table 3. The changes in the DHEAs of the Korean old female subjects before and after the performance of different types of the exercise program

		Means±SD	t	Sig	
AEC(N=0)	pre	40.0111±8.94964	7 4 4 4	.000***	
AEO(N-9)	post	74.5222±19.71996	-/.444		
DEC(N=0)	pre	47.8000±23.64017	0 177	.000***	
$\operatorname{KEO}(N-9)$	post	86.5444±36.32823	-0.1//		
SEC(N=0)	pre	53.2667±30.78559	0 262	.000***	
SEO(11-9)	post	100.2889 ± 40.74290	-8.303		

*p<.05, **p<.01, ***p<.001

Table 4. Changes in β-amyloid of the Korean old female compared among groups performing different types of exercise

0 1	<i>y y y</i>	1 00	110 00	<i>J</i> 1 <i>J</i>
	Sum of squares	df	F	Sig
between	.994	2	.364	.699
group total	32.781	24		
total	33.775	26		

*p<.05, **p<.01, ***p<.001

Table 5. Changes in DHEAs of the Korean old female compared among groups performing different types of exercise

	Sum of squares	df	F	Sig
between	10522.229	2	4.019	.031*
group total	31419.811	24		
total	41942.040	26		

*p<.05, **p<.01, ***p<.001

Table 6. Changes in DHEAs of the elderly female compared among groups performing different types of exercise

(I) group	(J) group	mean(I-J)	std.Error	Sig	post-hoc
AEG	REG	33.27778	17.05650	.171	
ALU	SEG	-13.74444	17.03030	.726	
DEC	AEG	-33.27778	17.05650	.171	DEC-SEC
KEU	SEG	-47.02222*	17.03030	.037	KEU~SEU
SEC	AEG	13.74444	17.05650	.726	SECNDEC
SEU	REG	47.02222*	17.03030	.037	SEO-KEU

*p<.05, **p<.01, ***p<.001

Discussion

The purpose of this study is to identify the changes in the β -amyloid, DHEAs and BDNF during 12 week period of exercise done by Korean elderly females subjects and to recognize the changes of variants for each type of exercise in order to provide useful information on dementia. We discuss the following based on the results of various variants.

Dementia is a disease where the brain function is hampered due to the accumulation of senile plaque, lumps of nerve fibers and amyloid in the brain tissues. It is reported that the rate of dementia differs according to the level of education, gender and genetic types and dementia is one of the most frequently occurring diseases around the world including Korea.(11-13) Therefore many researchers took interests in dementia and many studies were actively done on dementia. As a result the risk factors of dementia such as β -amyloid and DHEAs were identified. Elderly population showed the higher level of β -amyloid compared to the younger population and DHEAs, known to be related to the aging, would decrease over the time and is considered to be one of the risk factors of dementia. (14).

According to Park & Kwon's(15) study on the effect of mixed exercises on β-amyloid of elderly women, the comparison group showed tendency of increase in β -amyloid but the exercise group showed significant decrease. In Lee's(16) study on the effect of exercises on dementia risk factors of the elderly women, it is also reported that the control group showed gradual but significant increase in the level of β -amyloid from the first to the third set of the tests. However the experiment group showed significant reduction in the level of β-amyloid after 24 weeks of exercises compared to the pre-exercise stage. Also Cho, et al.(17) suggested that exercises were effective method of treating dementia based on his study with the subjects of NSE/PS2m-transgenic mice. In his study, those mice performed treadmill exercises everyday at a speed of 22cm per second for 30 minutes at the temperature level of 20~22°C for three months duration. The comparison group did not show any difference, but the exercise group showed significant reduction in β -amyloid and the exercise group also showed significant improvement in action function(way-finding). In the present study, for the purposes of reducing neuron senile plaque which is caused by lack of neurotransmitter, the subjects performed aerobic exercises, resistance exercises and sensory impact exercises where the stimulation of the neurons and physical activities were performed simultaneously. The result showed that each exercise group showed reduction of β -amyloid but there was no significant difference among groups. This is believed to be because of the duration of the exercises.

According to Lee(17)s study the level of β -amyloid suddenly dropped after 24th week of exercises compared to the level before the exercise began. And compared to the level during 12th week, the drop in the level after 24th week still suggests significant level. Therefore it is believed that among qualitative factors and quantitative factors which should be considered when an exercise is prescribed, the duration of the exercise should also be seriously taken into consideration.

DHEAs, which is one of the gonadal hormones produced in the adrenal, begins to decrease from puberty as people grow old and when they become elderly the level of DHEAs would be reduced to 25-33% of the level when they were young. However, the level of DHEAs is particularly low in the case of those with Alzheimer and vascular dementia, therefore DHEAs are suggested to be one of the dementia risk factors. (18).

When the previous studies on the resistance exercise were reviewed, the change in the level of DHEAs showed different results. For instance Hakkinen, K., et al(19) reported that when the middle aged and elderly subjects performed 6 months of resistance exercises, there was no change in the level of DHEA and DHEAs. However, the study done by Park and Kwon(15) showed different results when their elderly female subjects performed the muscular exercise for 12 weeks. DHEAs showed significant increase compared to the level before the exercise was performed. Also the studies done on the kinematical intervention on the changes in the level of DHEAs showed different results. It is reported that there was no change in the level of DHEA and DHEAs after 6months duration of resistance exercises done by the middle aged and elderly female subjects (19),

Some studies reported that when elderly subjects with the average age of 68.3 years were examined those with active lifestyle showed the significantly higher level of DHEAs compared to those with sedentary lifestyle.(20) Also it is reported that when the relationship between physical strength and DHEAs were explored among the elderly subjects with the age between 66 and 86, (25 male and 25 female), the male subjects did not show any significant relationship between physical strength and DHEAs but the female subjects showed the higher correlation between DHEA and the level of physical activities, maximum oxygen intake and muscular mass of their thigh. The conclusion was that for the female subjects the higher physical activities and the level of physical strength, the higher the level of DHEAs.

This study showed identical results with the previous studies and it is believed that this is because the subjects were elderly female.

As for the previous studies on the sensory impact exercise, Teri et al.,(21) reported that there was overall improvement in the physical functions and reduction in the level of depression and behavioral disorder when 30 subjects with Alzheimer performed walking, arms stretching, upright balance and flexibility related exercises for 12 weeks in order to improve physical function balance, flexibility, muscular strength and muscular endurance.

The present study adopted sensory impact exercises based on the previous studies and as a result there was increase in the level of DHEAs. This study applied sensory impact exercise program which focused more on the improvement on cognitive functions compared to the exercise programs adopted in the previous studies. Exercises such as mat exercise, trampoline, balance board, Gym ball, hula hoop, throwing and catching balls, hopping on one leg and holding out on one leg were performed and there was an increase in the motivation for improvement in the cognitive ability and basic physical strength. Similar to the previous studies, there was increase in the level of DHEAs.

Therefore we believe the 12 weeks duration of exercise program for each of AEG, REG and SEG would be effective. We confirm the reduction in β -amyloid and increase in DHEAs in each exer-

cise program and we believe they would greatly contribute to the improvement in dementia.

Conclusion

We conclude the AEG, REG, SEG showed decrease in β -amyloid, dementia risk factor and increase in DHEAs after 12 weeks duration of exercise program. Accordingly we believe that these kinds of exercise programs would have positive effects on the female hormones and it would also be effective in combating female menopause related diseases.

References

- 1. Goldspink, D.F. Ageing and activity: Their effect on the functional reserve of the health and vascular smooth and skeletal musclure. Ergonomics, 48(11-14_, 1334-1351, Review. 2005
- 2. National Bureau of Statistics South Korea(2005). Population Projections
- Shin, J. D., & Kim, W. K. Effects of taekwondo poomsae training on body composition, β-amyloid and dheas concentration in elderly women, Korean Alliance for Health Physical Education, 48(6), 503-511. 2009.
- 4. McDowell, L. Alzheimer' disease: Insights from epide-miology. Aging. 13(3), 143-162. 2001.
- 5. Huang, Y. Apolipoprotein E and Alzheim diesease. Leurology, 66(2 Suppl 1), S79-85. 2006.
- Van Praag, H., Kempermann, G., Gage, F.H.. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. Nature Neuroscience. 2(3), 266-270. 1999.
- 7. Barril, X., Orozco, M., & Luque, F. J. Towards improved acetylcholinesterase inhibitors; a structural and computational approach. Mini Review Medicine Chemistry, 1(3), 255-266. 2001.
- Bergeron, J., Charles, C., Jean, P.D., Jacques, G., Arther, S., Leon, D.C., Rao, J,S., Skinner, J., Willmore, H., Claude, B. Race differences in the response of postheparin plasma lopoprotein lipase and hepatic lipase activities to endurance exercise exercise training in men; result from the HERITAGE Familiy Study. Atherosclerosis, 159, 399-406. 2001.
- 9. ACSM. ACSM'S Gudeline for Exercise Testing and Prescription, 6th eds, Lippincott Williams and Wilins.117. 2001.
- 10. Karvonen, m and M. O. Kentalak. The dffects of train-ing heart rate, a longitudinal study. Arch. Intern. Med. 139, 857-962. 1979.

- 11. Price, D. L, Tanzi, R. E, Borchlet, D.R & Sisodia, S. S. Alzheimer's disease: genetic studies and transgenic models. Annu Rev Genet. 32, 461-93.
- 12. Selkoe, D. J.(2000). The genetic and molecular pathology of Alzheimer's disease: roles of amyloid and the presenilins. Neurol Clin, 18(4), 903-922. 1998.
- 13. Moceri, V. M., Kukull, W. A., Emanual, I., van Belle, G & Larson, E. B.. Early-life risk factors and the development of Alzheimer's disease. Neurology, 54(2), 415-420. 2000.
- Gray, A, Feldman, H. A, Mckinlay, J. B., & Longcope, C.. Age, disease, and changing sex hormone levels in middle-aged men: results of the Massachusetts Male Aging Study. J Clin Endocrinol Metab, 73(5), 1016-25. 1991.
- Park, S. K., &, Kwon, Y. C. The Effects of Combined Exercise on Respiratory Function, β-amyloid and DHEAs concentration in Elderly Women. Journal of Sports and Leisure Studies, 26, 257-268. 2006.
- 16. Lee, K. O.. The effects of the gymnastic exercise and walking program on body composition, depression and risk factors of dementia in the elderly women. Korean Alliance for Health Physical Education, 18(2), 1011-1026. 2009.
- Cho, J. Y., Hwang, D. Y., Kang, T. S., Shin, D. H., Hwang, J. H., Lim, C. H., Lee, S. H., Lim, H. J., Min, S. H., Seo, S. J., Song, Y. S., Nam, K. T., Lee, K. S., Cho, J. S., & Kim, Y. K., Use of NES/PS2m-transgenic mice in the study of the protective effect of exercise on Alzheimer's disease. J Sports Sci. 21(11), 943-951. 2003.
- Brown, R.C., Cascio, C., Papadopoulos, V. Neurosteroids: oxidative stressmediated dehydroepiandrosterone formation in Alzhrimer's disease pathology. Neurobiol Aging 21, S238. 2000.
- Hakkinen, K., Pakarinen, A., Kramer, W.J., Newton, R.U., Alen, M. Basal concentrations and acute responses of serum hormone and strength development during heavy resistance training in middle-aged and elderly men and women. J Gerontal A Biol Sci Med Sci. 55(2), B95-105. 2000.
- 20. Frisoni, G. B., Padovani, A., Wahlund, L.O. The Predmentia Diagnosis of Alzheimer Disease, Dis Assoc Disord. 18(2), 51-53. 2004.
- Teri, L., Gibbons, L.E., Mc Curry, S.M., Logsdon, R.G., Buchner, D.M., Barlow, W.E., Kukull, W.A., LaCroix, A.Z., McCormick, W., & Larson, E.B. (2003). Exercise plus behavioral management in patientswith Alzheimer'disease: a randomized controlled trial. JAMA, 290(15), 2015-2022.

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The effects of anticholinergic and alpha blocker combination therapy on male sexual functions and lower urinary tract symptoms in patients with BPH

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Abstract

Objectives/Background: Benign prostatic hyperplasia (BPH) is a common clinical condition that causes lower urinary tract symptoms. In patients with BPH, the overactive bladder symptoms can also be seen in addition to voiding and storage symptoms. The usage of an alpha-blocker and an antimuscarinic agent in combination seems to be convenient in such overactive bladder symptoms because overactivity of detrusor may develop due to hypersensitivity of detrusor muscles in BPH patients. The aim of this study was to evaluate the effects of alpha blocker (tamsulosin) and anticholinergic (propiverin) combination therapy on male sexual functions and lower urinary tract symptoms.

Methods: Between January 2009 and May 2010, a total of 91 patients with the diagnosis of BPH were retrospectively evaluated. Patients were randomly divided into two groups and in Group 1 (n=36) the patients were assigned to take both tamsulosin 0.4 mg plus propiverine 15 mg for 12 weeks. In Group 2 (n=55) the patients has got only tamsulosin treatment for a same period. In pretreatment evaluation the detailed medical history, physical examination, urinalysis, routine hematologic and biochemical analysis, serum prostatic specific antigen (PSA) level and uroflowmetry results of the patients were obtained. The International Index of Erectile Function Questionnaire (IIEF) and International Prostate Symptom Score (IPSS) of the patients were also recorded and urinary ultrasonography and cystoscopy were performed, if necessary. After three months of treatment all of the patients in both groups were evaluated with uroflowmetry, IPSS and IIEF scores and the results of these parameters before and after treatment were compared.

Results: The mean age of the patients were 64.36±9.65 years and 62.33±5.73 years in group 1 and group 2, respectively (p>0.05). In group 1, the mean IPSS scores decreased from 18.04±4.69 to 12.60±4.07 (p=0.001). In group 2, pre and posttreatment IPSS scores were found as 16.97±3.47 and 14.00±3.99, respectively (p=0.001). IPSS scores were significantly improved in both groups after treatment but the reduction in IPSS in combination group (Group I) was greater than tamsulosin group. The increase in mean Qmax values were 3.69 ml/sn and 2.77 ml/sn in Group 1 and Group 2, respectively (p=0.001). The pretreatment erectile dysfunction ratios were 41.8% (n=23) and 27.8% (n=10) in Group 1 and Group 2, respectively. After treatment these ratios were 34.5% (n=19) in Gropu 1 and 22.2% (n=8) in Group 2 (p>0.05). Premature ejaculations rates were 69.1% (n=38) and 15 (41.7% (n=15) in Group 1 and Group 2, respectively. After treatment premature ejaculation rates were also decreased in both groups (p>0.05).

Conclusion: As a result, it can be thought that the usage of propiverine and tamsulosin in combination was effective in patients with BPH and overactive bladder symptoms. However, it should also be added that randomized and controlled studies with larger series are needed to support these results.

Keywords: Prostate, propiverine, tamsulosin, overactivity, erectile dysfunction.

Introduction

Benign prostatic hyperplasia (BPH) is characterized as the nonmalignant hyperplasia of prostatic cells, which might eventually lead to bladder outlet obstruction (1). The clinical BPH is generally associated with various combinations of voiding and storage symptoms. In patients with BPH, overactive bladder symptoms can also be seen in addition to voiding and storage symptoms (2). In clinical practice, α-adrenergic receptor antagonists or 5-alpha reductase inhibitors are commonly used to treat lower urinary tract symptoms (LUTS) associated with BPH (3-5). Although the treatment with either an alpha1-adrenergic receptor antagonist or a 5-alpha reductase inhibitor provides improvement in symptoms, many patients with LUTS, which is associated with BPH, still suffer from the symptoms affecting their quality of life. It has been reported that overactivity in the bladder may be an important factor in the treatment failure in these patients (6). Recent studies have demonstrated the effectivity and safety of an antimuscarinic plus an alpha-antagonist combination therapy in improving both the urodynamic and patient-reported outcomes in men with LUTS and BPH, as well (7-10).

The aim of this study was to evaluate the effect of combination theraphy with an anticholinergic and an alpha blocker agent on lower urinary tract symptoms and male sexual functions in patients with BPH and overactive bladder symptoms.

Material and methods

Between January 2009 and May 2010 a total of 91 patients with the diagnosis of BPH were included to study. Patients were divided into two groups. In Group 1 (n=36) patients received propiverine 15 mg/day, p.o. and tamsulosin 0.4 mg/day, p.o. combination treatment and in Group 2 (n=55) patients received daily tamsulosin 0.4 mg (n=55) for 12 weeks. All patients were evaluated with detailed medical history, physical examination including digital rectal examination, urinalysis, routine hematologic and biochemical analysis and serum prostate specific antigen (PSA) levels, as well. Uroflowmetric studies and International Prostate Symptom Scores (IPSS) of all the patients were also obtained. If it was necessary urinary system ultrasonography and cystoscopy were performed. Before and at the end of the third month of treatment period, the erectile functions of the patients were evaluated with International Index of Erectile Function Questionnaire (IIEF), IPSS scores and uroflowmetric tests were performed. Changes in the IPSS and IIEF before and after treatment were compared. The eligible patient profile for the inclusion into the study was as follows: a men who was > 40 years old and had IPSS score > 12and micturition frequency of >8/day and had urgency symptoms. Exclusion criteria were history of surgical treatment for BPH, prostate biopsy within the last 6 months, use of 5 alpha-reductase inhibitors within 6 months, any urologic cancer, previous prostate or bladder/pelvic radiation history, urinary system stone disease, active urinary system infection, acute urinary retention in the last 6 months, acute or chronic hepatic or renal failureand neurologic disorders affecting the micturation process. The patients with clinically significant bladder outlet obstruction or serum PSA of more than 10 ng/mL with risk of prostate cancer were also excluded from the study.

Statistical Analysis

Pearson Chi-square and McNemar tests (for independent and dependent groups respectively) were used to compare the categorical variables . The categorical variables were presented as count and percentages. All continuous variables had normal distribution according to Kolmogorov Smirnov normality test. Therefore, two independent sample t test was used to compare the continuous variables between BPH+urge incontinence and BPH groups; two paired sample t test was used to compare the pretreatment and post treatment mesurements. The two way ANOVA was used to compare the alterations of the continuous variables during the treatment procedure between BPH+urge incontinence and BPH groups. The continuous variables were presented as mean and standard deviation. A p value of <0.05 was considered statistically significant. Analyses were performed using commercially software (PASW ver.18, ID:33478001 SPSS inc. Chicago, IL)

Results

The mean age of the patients were 64.36 ± 9.65 years and 62.33 ± 5.73 years in Group 1 and Group 2, respectively (p>0.05). In Group 1, the total IPSS decreased from 18.04 ± 4.69 to 12.60 ± 4.07 (P=0.001). In Group 2, pretreatment and posttre-

atment IPSS scores were found as 16.97±3.47 and 14.00 ± 3.99 , respectively (P=0.001). The changes from baseline in the two groups after treatment with tamsulosin or the combination of tamsulosin plus propiverine for 12 weeks were presented in Table 1. The IPSS were significantly improved in both groups after treatment, and the reduction of IPSS in the combination group was greater than that in the tamsulosin group (Figure 1). The increase in Qmax were 3.69 ml/sn and 2.77 ml/sn in Group 1 and Group 2, respectively (P=0.001). The difference between the increases in Qmax values of the two groups was not statistically significant (p=0.119) (Figure 2). The erectile dysfunction rates were 41.8% (n=23) and 27.8% (n=10) in Group 1 and Group 2, respectively. After treatment these ratios were 34.5% (n=19)) and 22.2% (n=8) in Group 1 and Group 2. Premature ejaculations



Figure 1. Pre and posttreatment IPSS scores of the groups

rates were 69.1% (n=38) and 41.7% (n=15) in Group 1 and in Group 2, respectively. After treatment there was no statistically significant decrease in the premature ejaculation rates. Painful ejaculation rates were decreased in both groups after the treatment. The incidence of urinary retention and voiding difficulties was low in Group 2 (n=1) in comparison to Group 1 (n=2) (Table 1).

Discussion

Benign prostatic hyperplasia is a common disorder among middle-aged and elderly men. Approximately 50% of men aged 50 to 60 years, 60% of men aged between 60-70years and up to 90% of men aged >80 years have some degree of BPH (11). The pathology is characterized histologically by cell proliferation in the transition zone



Figure 2. The changes in Qmax values of the groups

		Group 1	p ¹	Group 2	p ¹	p ²	p ³
Age (y	year)	64.36±9.65		62.33±5.73		0.561	
IDCC*	Pre-Treatment	18.04±4.69	0.001	16.97±3.47	0.001	0.217	0.942
11.22	Post-Treatment	12.60 ± 4.07	0.001	14.00±3.99	0.001	0.110	0.045
Uroflowmetry,	Pre-Treatment	14.95 ± 4.89	0.001	13.92±3.84	0.001	0.289	0.110
ml/sec*	Post-Treatment	18.64±6.45	0.001	16.69±4.01	0.001	0.080	0.119
ED $m(0/)$	Pre-Treatment	23 (41.8)	0.125	10 (27.8)	0.500	0.255	
ED, II (70)	Post-Treatment	19 (34.5)	0.123	8 (22.2)	0.300	0.306	-
Premature	Pre-Treatment	38 (69.1)	0.0(2	15 (41.7)	0.275	0.017	
Ejaculation, n (%)	Post-Treatment	33 (60.0) 0.06.		12 (33.3)	0.375	0.023	-
Painful	Pre-Treatment	5 (9.1)	0.050	2 (5.6)	1 000	0.699	
Ejaculation, n (%)	Post-Treatment	2 (3.6)	0.250	1 (2.8)	1.000	1.000	-

Table 1. The clinical and demographic charecteristics of the groups

* Values were presented as mean \pm standard deviation.

1: The results of comparisons between pretreatment and post treatment measures separetely for groups.

2: The results of comparisons between BPH+urge incontinence and BPH groups.

3: The results of comparisons of alteration for pre and post treatment between BPH+Urge Incontinence and BPH groups.

of the prostate (static component), and physiologically by increased tone of the smooth muscle in the prostate and bladder neck region (dynamic component) leading to LUTS (1,3). The LUTS are divided into three categories as storage, voiding, and postmicturition symptoms. The storage/ filling symptoms are bothersome for most of the patients and negatively impact their daily activities and have adverse effects on the quality of life (12). In the treatment of BPH several modalities are avaliable including watchful waiting, pharmacotherapy, minimally invasive therapies, transurethral prostate resection, and open prostatectomy (13,14). First-line pharmacologic treatment options for men with BPH are alpha-blockers such as alfuzosin, doxazosin, tamsulosin, or terazosine and the 5-alpha reductase inhibitors. Alpha-blockers act on the dynamic component of obstruction by blocking smooth muscle contraction in the prostate and bladder neck, resulting in the relaxation of the bladder neck, improvement in urinary flow rate, and an amelioration of LUTS generally (14). The effectivity of the alpha blockers have been reported in numerous clinical studies in patients with BPH (15,16).

Detrusor overactivity may coexist with bladder outlet obstruction due to BPH. This condition is characterized by urinary urgency, frequency and nocturia with or without urge incontinence. Several studies has explained the causal relationship between BPH and the development of bladder overactivity. One of them was bladder wall hypertrophy due to overexertion secondary to bladder outlet obstruction which was associated with progressive detrussor denervation. It has been postulated that the areas of detrussor denervation might develop hypersensitivity to acetylcholine, which might be one of the causes of bladder overactivity, as well as the unregulated release of acetylcholine from the damaged neuronal plexus. Alpha-blockers act mainly on the voiding symptoms caused by obstruction but they have minimal effects on the filling symptoms caused by bladder dysfunction. Therefore, if the patients have mixed overactive bladder symptoms and BPH, antimuscarinic and alpha-blocker combination can be used in the treatment of BPH (14). Antimuscarinics are competitive antagonists of muscarinic receptors on the detrusor muscle which act by blocking cholinergic stimulation. During bladder filling, these drugs suppress involuntary detrusor contractions caused by basal release of neuronal and urothelial acetylcholine (17,18). Thus, due to the decrease in the feeling of urgency to empty the bladder micturition activity is delayed. Several antimuscarinic agents are available for the treatment of overactive bladder symptoms such as oxybutynin, tolterodine, solifenacin, darifenacin, trospium chloride, and fesoterodine. Although the drugs may differ in molecular structure, molecular size and muscarinic receptor selectivity, they all share the same mechanism of action which is the prevention of acetylcholine, release from the parasympathetic presynaptic nerve terminal and inhibition of binding to the M3 mucscarinic receptor in the detrusor (19). The efficacy of these drugs have been reported in many previous clinical and experimental studies (20-22). In this context, Lee et al reported that 32 (73%) of 44 men with urodynamically confirmed detrusor overactivity and bladder outlet obstruction who did not respond to treatment with doxazosin experienced symptomatic improvements after 12 weeks of treatment with doxazosin and tolterodine (23). In another clinical study reported by Athanasopoulos, 50 consecutive patients were randomized to tamsulosin plus tolterodine or only tamsulosin treatment for 12 weeks. The patients treated with combination of an alpha-blocker and antimuscarinics experienced significant reduction in maximum detrusor pressure during micturition, significant increase in bladder capacity and first unstable contraction occurred in higher volumes urodynamically (24). Thus authors suggested that the combination treatment with an alpha-blocker plus an anticholinergic improved quality of life in patients with bladder outlet obstruction and treated the detrusor instability, concomitantly. In another prospective study, Kaplan et al revealed the efficacy of extended release tolterodine in men with LUTS who had failed previous alpha-blocker therapy (25). In our study, antimuscarinic and alpha-blocker combination also resulted in beneficial clinical conditions. In a prospective study, Yang et al. randomized 69 men on terazosin or a combination regimen consisting of terazosin and tolterodine. While IPSS scores improved significantly for both groups at 6 weeks, the improvement in the combination group

was significantly greater (26). It can be speculated that, combination theraphy may cause urinary retention, but in previous randomized trials it has been shown that the incidence of urinary retention with active treatment was comparable with placebo (27,28).

Erectile dysfunction (ED), defined as the inability to achieve or maintain an erection sufficient for satisfactory sexual performance, is also a common clinical disorder. Depending on age the reported prevalence of ED varies between 52% and 80% in men aged 40 to 80 years (29,30). Similarly in a study, the age-adjusted overall prevalence of ED in Turkey was 69.2% (mild 33.2%, moderate 27.5%, severe 8.5%) and increased with age, as did severity of ED. When authors consider moderate + severe ED cases, the prevalence was 36% (31). Erectile dysfunction is associated with several recognized risk factors such as hypertension, heart disease, aging, obesity, dyslipidemia, diabetes mellitus, hypogonadism, pelvic surgery, smoking, psychiatric disease, medications and LUTS (32-34). It has been reported that the presence and severity of LUTS is associated with sexual dysfunction and decreased sexual activity and satisfaction (35-37). The relationship between voiding symptoms and sexual dysfunction were evaluated in many previous studies (38). A few studies have pointed out that the storage symptoms, those signified overactive bladder symptoms, were highly associated with sexual dysfunction (39). Irwin et al. performed a case-control study of 502 men with overactive bladder and 502 age matched controls (39) Significantly more cases (14%) in the overactive bladder group reported reduced sexual activity because of urinary symptoms compared with controls (4%).

There is limited information in the literature regarding the use of anticholinergics and male sexual symptoms. In a study, Temml et al. provided same data, in which the sexual function, as well as overactive bladder symptoms, improved significantly in patients who were treated with transdermal oxybutynin during six month (40). Sand et al. confirmed this assumption, as well and they reported that the most significant factor for improving sexual quality of life was the reduction of coital incontinence as a consequence of the anticholinergic treatment of the detrusor overactivity (41). In present study, the erectile dysfunction rates decreased in both groups after treatment.

Conclusions

In conclusion, the limitations of this study should be mentioned which were the small scale of the study and the absence of urodynamically proven obstructive status of the participating patients. But it can be added that further randomized studies with larger series are needed to support our results and enlightenment of this issue.

References

- 1. Roehrborn CG. Male lower urinary tract symptoms (LUTS) and benign prostatic hyperplasia (BPH). Med Clin North Am 2011; 95:87-100.
- 2. Kaplan SA, Roehrborn CG, Abrams P, et al. Antimuscarinics for treatment of storage lower urinary tract symptoms in men: a systematic review. Int J Clin Pract 2011; 65:487-507.
- 3. Lepor H, Kazzazi A, Djavan B. α-Blockers for benign prostatic hyperplasia: the new era. Curr Opin Urol 2012; 22:7-15.
- 4. Biester K, Skipka G, Jahn R, et al. Systematic review of surgical treatments for benign prostatic hyperplasia and presentation of an approach to investigate therapeutic equivalence (non-inferiority). BJU Int 2012; 109:722-730.
- Sun J, Xiang H, Yang LL, et al. A review on steroidal 5α-reductase inhibitors for treatment of benign prostatic hyperplasia. Curr Med Chem 2011; 18:3576-3589.
- 6. Chung SD, Chang HC, Chiu B, et al. The efficacy of additive tolterodine extended release for 1-year in older men with storage symptoms and clinical benign prostatic hyperplasia. Neurourol Urodyn 2011; 30:568-571.
- 7. Rovner ES, Kreder K, Sussman DO, et al. Effect of tolterodine extended release with or without tamsulosin on measures of urgency and patient reported outcomes in men with lower urinary tract symptoms. Urol 2008; 180:1034-1041.
- 8. Athanasopoulos A, Perimenis P. Efficacy of the combination of an alphal-blocker with an anticholinergic agent in the treatment of lower urinary tract symptoms associated with bladder outlet obstruction. Expert Opin Pharmacother 2005; 6:2429-2433.

- 9. Bae JH, Kim SO, Yoo ES, et al. Efficacy and safety of low-dose propiverine in patients with lower urinary tract symptoms/benign prostatic hyperplasia with storage symptoms: a prospective, randomized, singleblinded and multicenter clinical trial. Korean J Urol 2011; 52:274-278.
- Greco KA, McVary KT. The role of combination medical therapy in benign prostatic hyperplasia. Int J Impot Res 2008; 20:33-43.
- 11. Roehrborn CG ve McConnell JD: "Etiology, pathophysiology, epidemiology, and natural history of benign prostatic hyperp-lasia". In Campbell's Urology, 2002;1297-1330
- 12. Tikkinen KA, Tammela TL, Rissanen AM, et al. Is the prevalence of overactive bladder overestimated? A population-based study in Finland. PLoS One 2007; 2:195.
- 13. Juliao AA, Plata M, Kazzazi A, et al. American Urological Association and European Association of Urology guidelines in the management of benign prostatic hypertrophy: revisited. Curr Opin Urol 2012; 22:34-39.
- Lepor H, Kazzazi A, Djavan B. α-Blockers for benign prostatic hyperplasia: the new era. Curr Opin Urol 2012; 22:7-15.
- 15. Dutkiewicz S, Witeska A. Doxazosin--an alpha-1 receptor blocking agent in the long-term management of benign prostatic hyperplasia (Part One). Int Urol Nephrol 1995; 27:311-318.
- 16. Lloyd SN, McMahon A, Muller W, et al. Comparative study of selective alpha 1-adrenoceptor blockade versus surgery in the treatment of prostatic obstruction. Br J Urol 1994; 73:723.
- 17. Karram MM, Toglia MR, Serels SR, et al. Treatment with solifenacin increases warning time and improves symptoms of overactive bladder: results from VENUS, a randomized, double-blind, placebo-controlled trial. Urology 2009; 73:14-18.
- 18. Chapple CR, Khullar V, Gabriel Z, et al. The effects of antimuscarinic treatments in overactive bladder: an update of a systematic review and meta-analysis. Eur Urol 2008;54:543-562.
- 19. Ellsworth P. Treatment of overactive bladder symptoms beyond antimuscarinics: Current and Future Therapies. Postgrad Med 2012; 124:16-27.
- 20. MacDiarmid SA, Peters KM, Chen A, et al. Efficacy and safety of extended-release oxybutynin in combination with tamsulosin for treatment of lower urinary tract symptoms in men: randomized, double-blind, placebo-controlled study. Mayo Clin Proc 2008; 83:1002-1010.

- 21. Sugaya K, Nishijima S, Tasaki S, et al. Effects of propiverine and naftopidil on the urinary ATP level and bladder activity after bladder stimulation in rats. Neurosci Lett 2007; 429:142-146.
- 22. Nitti VW, Dmochowski R, Sand PK, et al. Efficacy, safety and tolerability of fesoterodine for overactive bladder syndrome. J Urol 2007; 178:2488-2494.
- 23. Lee JY, Kim HW, Lee SJ, et al. Comparison of doxazosin with or without tolterodine in men with symptomatic bladder outlet obstruction and an overactive bladder. BJU Int 2004; 94:817-820.
- 24. Athanasopoulos A, Gyftopoulos K, Giannitsas K, et al. Combination treatment with an alpha-blocker plus an anticholinergic for bladder outlet obstruction: a prospective, randomized, controlled study. J Urol 2003; 169:2253-2256.
- 25. Kaplan SA, Walmsley K, Te AE. Tolterodine extended release attenuates lower urinary tract symptoms in men with benign prostatic hyperplasia. J Urol 2008; 179:82-85.
- 26. Yang Y, Zhao XF, Li HZ, et al. Efficacy and safety of combined therapy with terazosin and tolterodine for patients with lower urinary tract symptoms associated with benign prostatic hyperplasia: a prospective study. Chin Med J (Engl) 2007; 120:370-374.
- 27. MacDiarmid SA. Combination antimuscarinics and alpha-blockers for benign prostatic hyperplasia. Curr Urol Rep 2008; 9:265-271.
- 28. Chung SD, Chang HC, Chiu B, et al. The efficacy of additive tolterodine extended release for 1-year in older men with storage symptoms and clinical benign proastatic hyperplasia. Neurourol Urodyn 2011; 30:568-571.
- 29. Travison TG, Shabsigh R, Araujo AB, et al. The natural progression and remission of erectile dysfunction: results from the Massachusetts Male Aging Study. J Urol 2007; 177:241-246.
- 30. Safarinejad MR, Hosseini S. Erectile dysfunction: clinical guidelines. Urol J 2004; 1:133-147.
- 31. Akkus E, Kadioglu A, Esen A, et al. Turkish Erectile Dysfunction Prevalence Study Group. Prevalence and correlates of erectile dysfunction in Turkey: a population-based study. Eur Urol 2002; 41:298-304.
- 32. Berrada S, Kadri N, Mechakra-Tahiri S, et al.Prevalence of erectile dysfunction and its correlates: a population-based study in Morocco. Int J Impot Res 2003; 15:1:3-7.
- *33.* Seftel AD. Erectile dysfunction in the elderly: epidemiology, etiology and approaches to treatment. J Urol 2003; 169:1999-2007.

- 34. Kubin M, Wagner G, Fugl-Meyer AR. Epidemiology of erectile dysfunction. Int J Impot Res 2003; 15:63-71.
- 35. Ströberg P, Boman H, Gellerstedt M, et al. Relationships between lower urinary tract symptoms, the bother they induce and erectile dysfunction. Scand J Urol Nephrol 2006; 40:307-312.
- 36. Reggio E, de Bessa J, Junqueira RG, et al. Correlation between lower urinary tract symptoms and erectile dysfunction in men presenting for prostate cancer screening. Int J Impot Res 2007; 19:492-495.
- 37. Glina S, Santana AW, Azank F, et al. Lower urinary tract symptoms and erectile dysfunction are highly prevalent in ageing men. BJU Int 2006; 97:763-765.
- 38. Wein AJ, Coyne KS, Tubaro A, et al. The impact of lower urinary tract symptoms on male sexual health: EpiLUTS. BJU Int 2009; 3:33-41.
- 39. Irwin DE, Milsom I, Reilly K, et al. Overactive bladder is associated with erectile dysfunction and reduced sexual quality of life in men. J Sex Med 2008; 5:2904–2910.
- 40. Temml C, Haidinger G, Schmidbauer J, et al. Urinary incontinence in both sexes: prevalence rates and impact on quality of life. Neurourol Urodyn 2000; 19:259–271.
- 41. Sand PK, Goldberg RP, Dmochowski RR, et al. The impact of the overactive bladder syndrome on sexual function: a preliminary report from the Multicenter Assessment of Transdermal Therapy in Overactive Bladder with Oxybutynin trial. Am J Obstet Gynecol 2006; 195:1730-1735.

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The efficiency of based on mind fullness cognitive therapy upon depression, anger, obsessive rumination in dysthymic patients

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Abstract

The aim of this study was the determining of the efficacy of mind fullness therapy based on cognitive therapy upon depression, anger and obsessive rumination in dysthymic patients. The statistical society of this research included 200 persons of inferring patients to psychological clinic in Esfahan which 110 persons of them, the persons who their scares was higher (more) than the cut - offpoint for executed questionnaires like depression, anger and obsessive rumination. 40 persons were selected by random sampling among them and put into two experimental groups and one control group. The research's design was experimental and pretest - posttest with control group. After random selecting of experimental and control groups, it was executed pretest for both groups, then it was performed experimental intervention of mindfulness based on cognitive therapy upon experimental groups and after completing this program, it was done posttest. The results gained from the study using covariance analysis revealed that the research hypothesis has been accepted (confirmed) on the basis of P < 0/01.

Keywords: Depression, anger, obsessive rumination, dysthymic health organization.

Introduction

Recently, one design of world has predicted that the depression would be the second disorder among all disorders at 2010 (Segal & Williams, 2002). The dysthymia disorder is a chronic disorder that is known by depressed mood for most dags. This disorder is a prevalent disorder among people. The prevalence rate is almost 6% in life's period and its yearly prevalence rate is almost 3% for all people. More than 36% of psychiatric out patients show depression too. The chronic depression is known by longer therapy period, low physical healthy, more associated disorders, and more acute disorders in social, emotional and psychological performance than serve depression. The persons with this disorder use healthy care systems more than the usual persons and also show more suicide and being confined to bed. (kriston wolff,2010)

The response style of obsessive rumination: the obsessive responses are defined as repeated thoughts and also the behaviors that the depression symptoms, reasons and its results makes important for depressed person. The obsessive rumination is known as unwanted process of passive and negative thoughts attack while makes important the depression symptoms and their meaning (lyubomirsky & collageous, 1995).

Anger hot temper and aggression are used erroneously because they are supposed similar to each other. In spite of this fact, we can define the anger as on emotional state or internal feeling raised from physiological arousal, cognition and the thought related to vengeance (O'Neill, 2006).

In order to separate these three concepts, we can know anger as emotion, vengeance as attitude and aggression as behavior. Anger is defined as an emotional state that comprises the foundation of vengeance and aggression. The purpose form vengeance is an aggressive attitude which leads person toward aggressive behavior, while aggression has been knows as a observable behavior that is done with the aim of making damage (Del Vecchio o'leary ,2004).

Mindfulness based on cognitive therapy (MBCT) is a psychological therapy that combines the aspect of Beck's cognitive therapy and the

program of decreasing stress based on mindfulness of ,(kabat-zinn 1990) and is shown by emphasizing upon the present time and an nonjudgeable knowledge toward internal and external experiences. In this method, unlike beck's cognitive therapy, no Endeavour is devoted for correcting cognitive errors and expelling some thoughts from consciousness (Segal & Williams, 2002).

One main hypothesis about this method is that mind processes the experiences by two methods. One method which is called "performance" method, tries to decrease the distance between the present state with the wanted state by continuous solving problem and the other method is called " being " or " understanding " method that doesn't try to change natural situations. The first method is applicable about many life fields but it is changed into unsuitable state in the thought of depressed persons, therefore the aim of mindfulness is the changing of thinking style toward " being " method. The aim of this therapy method is that it can instruct one method to the patients until they can encounter with their thoughts and experiences differently (Crane, 2009).

So that person receive mindfulness skills, learns that it's better to suppose low power, will and importance for his/her judgment and reproach because they are a basic source for improving the negative thoughts and then he/she can react to these state kindly (kuyken,2010).

The aim of the mindfulness therapy based on cognitive therapy is that they can understand that thoughts are alone thought and aren't "themselves" or realities. When they can look to their thoughts, merely as one thought and separated something form them, and then they can look with more clear sight to reality and have more management (control) upon realities by this therapy method.

Persons can learn attention, concentration and some techniques for practicing this concentration and its using in life and then makes them able to control themselves with appearing the first symptoms like negative mood, negative thoughts and the methods of un effect processing and then finally can be prevented from becoming (being) depressed by concentration upon breathing , physical emotions and their actions (Segal & Williams, 2002).

Method

The present research is an experimental study, the research design is pre test- post test design with control group that has been comprised of two group subjects and both two groups are assessed twice. At the present research, 110 persons among 200 patients were selected on the basis of the sample size and then were given these questionnaires to them (depression, aggression, obsessive rumination). After the first screening, 40 persons were selected among the persons who had the scores, higher than cut – off point and then were replaced in two experimental and control groups, each group comprising 20 persons. The experimental group received mindfulness therapy based on cognitive therapy at 8 sessions, each session, 2 hours. The control group received no therapy. After 8 weeks therapy, all persons of experimental and control group completed depression, aggression and obsessive rumination questionnaires again and it was gathered the necessity data and finally they were analyzed by SPSS software. The method of executing the mindfulness therapy sessions based on cognitive therapy: At this research, it was used of mindfulness therapy based on cognitive therapy which it comprises some techniques like eating raisin physical audit "seeing " and " listening" technique, and seating in meditation manner for mindfulness to sounds and thoughts , paying attention to the way of communicating with our experiences through reaction to our thoughts, emotions and physical senses and also can know thoughts as mere thought, not reality some exercises for discovering the relationship between activity and mood, preparing a list of enjoyable activities and some activities which give control feeling to person. These techniques are instructed for two months at 8 sessions, each session 2 hours once a week.

Measurement tool

For evaluating research variables, it is used of some questionnaires like Beck depression questionnaire, anger questionnaire (AQ), Noolen – Hakseme depression.

Data analysis

Main hypothesis: mindfulness therapy based on cognitive therapy is effective upon depression, anger and obsessive rumination decreasing of dysthymia patients. Eta square is known as a partial value of variance that is related to new compound variable. At the present study, Eta square includes depression, obsessive rumination and anger rate which we can select psychic well – being for new compound variable, its value is 0.931 that indicates effect value for mindfulness therapy training based on cognitive therapy and control group. The calculated effect value higher than 0.14 indicates the fit effect of mindfulness therapy training based on cognitive therapy upon depression, obsessive rumination and anger. The mindfulness therapy training based on cognitive therapy can manage depression, obsessive rumination and anger rate.

On the basis of acquired data of Table 4, it has been used of BenFeruni standardized Alpha (0.003) for two experimental and control groups.

After adjusting of depression pretest mean (26.70) , also with regarding acquired data form Table 2 for depression variable, and the calculated F, $\eta X = 0.781$, P= 0.001, (df= 1.35)=124.854, the calculated F is significant statistically because the significance level is low than Benferuni alpha (0.003). Therefore we can state that there is a significant difference between depression post test scores for two groups of mindfulness therapy based on cognitive therapy and control group. After adjusting the pretest mean of obsessive rumination (27.72) also with regarding acquired data from table 2 for obsessive rumination variable, and the calculated F , $\eta 2 = 0.828$, P = 0.001 , (df = 1.35) =168.533, is significant statistically because its significance level is low than Benferuni standardized Alpha (0.003). Therefore we can state that there is a significant difference between the obse-

Table 1. The score mean and standard deviation for pretest – posttest, the rates of depression, obsessive rumination and anger separately the mindfulness therapy group on cognitive therapy and control group

mindfulness therapy experimental group based on control group Variables									
Cognitive therapy	mean (X)	standard	deviation (S)	mean (X) deviation standard					
Depression posttest	26.15	4.23	27.25	2.82					
Depression posttest	15.60	3.02	26.15	2.75					
Obsessive rumination pretest	27.05	3.01	28.40	2.01					
Obsessive rumination posttest	17.80	3.07	27.40	2.01					
anger pretest	27.35	2.83	28.95	2.43					
anger posttest	17.95	2.68	27.95	2.43					

Table 2. The scores of adjusted mean and standard deviation the depression , obsessive rumination and anger rate for mindfulness based on cognitive therapy group and control group

Variables mindfulness therapy based on cognitive therapy control group						
therapy	mean (X)	standard	deviation (S)	mean (X) SD		
depression posttest	15.682	0.632	26.06	0.623		
Obsessive rumination posttest	18.30	0.440	26.89	0.449		
Anger posttest	18.49	0.293	27.40	0.293		

Table 3. Effect rate (Eta) based on lambda Wilks test for compound variable

Variable	value	F	df1	df2	significance	level Eta	test potentiality
group	%69	148.408	3	33	0.001	%931	1.000

Table 4. The analysis of covariance data for depression, obsessive rumination and anger variable

	SS(sum of square)	Df	MS	F	Р	Eta	Test potency
Depression	912.754	1	912.754	124.854	0.001	0.781	1.000
Obsessive rumination	623.924	1	623.924	0.533	0.001	0.828	1.000
Group anger	672.772	1	672.772	0.387	0.001	0.924	0.001

ssive rumination posttest scores for mindfulness therapy training based on cognitive therapy and control groups. After adjusting anger pretest mean (27.72), also, with regarding acquired data from table 2, for anger variable , and the calculated F, $\eta 2=0.924$, P = 0.001, (df = 1.35) = 426.317, is significant statistically because its significance level is low than Benferuni standardized Alpha. Therefore we can state that there is a significant difference between anger posttest scores for mindfulness therapy training based on cognitive therapy and control groups.

With paying attention to being significant (meaningful) the difference of means, we can state with 995 confidence that the research hypothesis, "mindfulness therapy based on cognitive therapy is effective upon decreasing depression, anger, obsessive rumination of dysthymic patients is confirmed.

Discussion and Conclusion

As it was started, the aim of this research was confirming the efficiency of mindfulness therapy based on cognitive therapy upon depression, anger and obsessive rumination of dysthymic patients.

The tables data indicated that there is a significant difference (P < 0.001) between the patients who received mindfulness based on cognitive therapy than the patients of control group in depression, anger and obsessive rumination and this means that mindfulness therapy on cognitive therapy is effective upon decreasing depression, anger and obsessive rumination of dysthymic patients.

Omidi and Collageous (2008) compared the combination of cognitive therapy based on mindfulness and cognitive – behavioral therapy with alone cognitive therapy and also using of drug usual therapies for persons who were acute depressed, the data were indicating the preference of two psychotherapy methods than drug therapy for decreasing depression symptoms , while there wasn't a significant difference between the efficacy of these two methods. Omidi and Collageous (2009) studied the efficacy of compound therapy, cognitive therapy based on mindfulness and cognitive – behavioral therapy upon decreasing the generalizability of long – term memory for depressed patients. The data of this study indicated that compound therapy was effective on decreasing the generalizability of long- term memory and increasing of its specializing than cognitive therapy and also drug therapy. Kavyani and Collageous (2008) studied the efficacy of cognitive therapy upon mindfulness upon depression among students. At one study, the students who received high scores from Beck's questionnaire, after receiving this training and at one survey including 60 days, their depression, anxiety, un effective attitude and autonomous thoughts was decreased (Kavyani & Collageous, 2005).

At the other study, this method had, increasing effect upon life's quality of depressed persons. Hanasabzade Isfahani (2008) indicted the efficacy of cognitive therapy upon mindfulness upon suicide thoughts, negative autonomous thoughts and depression of persons affected with major depression disorder. Hamidpoor (2007) studied the efficacy of MBCT upon therapy and preventing of occurring of the data indicated significant decrease upon the severity of depression anxiety and increasing of self - esteem of participants. William Rushell (2010) indicated the significant efficacy of mindfulness based on cognitive therapy in comparison with psychic training and also usual therapy for the patients with suicide thoughts, wellbeing from depression in preventing from recurring. Using of this method resulted in decrease of interfered anxiety of bipolar patients and also a significant decrease the scores of Beck depression and anxiety questionnaire of patients after therapy. Barenhofer (2009) studied the efficacy of this method in comparison with usual therapy upon patients with depression symptoms and previously had experienced one period of depression and also had suicide in one controlled survey. The data indicated decrease of depression remaining symptoms upon MBCT group. Keim & Keim (2010) used mindfulness therapy based on cognitive therapy upon the patients affected with panic and inclusive anxiety that 6 month using drug hadn't result in their wellbeing. Cognitive therapy based on mindfulness in composition with previous drugs than control group resulted in significant decrease of depression and anxiety which were measured with depression and anxiety questionnaire orderly. Keni & Williams (2006) used MBCT for the patients who were at active

(acute) period of depression and also were resistible against the other therapies. At the final stage, the depression scores of Beck questionnaire which was serer (high), decreased into mild until average. They also compared mindfulness (mentality knowledge) based on cognitive therapy (MBCT) with cognitive- behavioral therapy (CBT) upon two groups of non melancholic depressed patients who were passing from one period of depression. The acquired data indicated a significant decrease upon anxiety and depression scores for two groups and also no significant difference between these two therapies. Though these resulting were confirmed about the patients who had passed 4 periods and or low than it, previously, they are also congruent with the acquired data from the patients with more than 4 periods of depression based on being more effective cognitive therapy than mindfulness based on cognitive therapy.

References

- 1. Keyvani, Hossein, Hatami, Neda and Shafiabadi, Abdollah "the effect of cognitive therapy based on mindfulness upon life quality of depressed persons (nonclinical). The Journal of New Cognitive Sciences.4: 39-48. 2008.
- 2. Omidi, Abdollah, Mohammakhani, Parvaneh, Doolatshhi, Parviz, Poorshahbaz, Abbas. The efficacy of compound therapy of mind presence based on cognitive therapy and cognitive s- behavioral therapy upon decreasing generalizability of memory upon persons affected with major depression disorder. The Research Journal of Behavioral Sciences.2:116.2009.
- 3. Segal ZV, Williams JMG, Teasdale JD.Mindfulness based cognitive therapy for depression. New York, London. The Guilford press. 2002.
- 4. Pepper C.M, Klein D.N, Anderson R.L,Riso L.P,Ouimette P.C,& Lizardi H. DSM-III-R axis II comorbidity in dysthymia and major depression. Am j psychiatry. 152:239-247.1995.
- 5. Sadock B.J, Sadock V.A, & Ruiz P. Comprehensive textbook of psychiatry. Philadelphia, Baltimor, New York. LWW.2009.
- 6. Godfrin K.A, Heeringen C.V. The effects of mindfulness-based cognitive therapy on recurrence of depressive episodes, mental health and quality of life: A randomized controlled study. Behavior Research and Therapy.48:738-746.2010.

- 7. Chiesa A, Serretti A. Mindfulness based cognitive therapy for psychiatric disorders: A systematic review and meta-analysis. Psychiatry Research .2010.
- 8. Williams JMG, Russell LT, Crane C, Russell D, Whitaker CJ, Duggan DS, Barnhofer T, Fennell MJV, Crane R, & Silverton S. Staying well after depression: trial design and protocol. BMC Psychiatry.2010.
- 9. Barnhofer T, Crane C, Hargus E, Amarasinghe M, Winder R, & Williams JMG. Mindfulness-based cognitive therapy as a treatment for chronic depression: A preliminary study. Behaviour Research and Therapy .2009.
- 10. Kim B, Lee S, Kim YW, Choi TK, Yook K, Suh SY, Cho SJ,& Yook KH. Effectiveness of a mindfulnessbased cognitive therapy program as an adjunct to pharmacotherapy in patients with panic disorder. Journal of Anxiety Disorders .2010.
- 11. Manicavasgar V, et al. Mindfulness-based cognitive therapy vs cognitive behaviour therapy as a treatment for non-melancholic depression. J. Affect Disorder. 2010.
- 12. Nicolet, I. A.. The second step violence prevention program: Effectiveness of a brief social Skills Curriculum with elementary – age children. 2004.
- 13. Vitaro F, Pelletier D. Assessment of children's social problem-solving therapy for depression in children, abnormal psychology.15: 1-2. 1991.
- 14. Chang, E. C. Distinguishing between ruminative and distractive responses in dysphoric college students: Does indication of past depression make a difference? Personality and Individual Differences.36:845-855. 2004.
- 15. Moulds, M.c., Kandris, E., Starr, S., & Wong, A. C. M. The relationship between rumination, avoidance and depression in a non-clinical sample. Behaviour research and therapy.45:251-261. 2007.
- Nolen-Hoeksema, S. The role of rumination in depressive disorders and mixed anxiety/ depressive symptom. Journal of Abnormal Psychology.109 (3):504–511. 2000.
- 17. Nolen-Hoeksema, S., Wisco, B. E., & Lyubomirsky, S. Rethinking Rumination. Perspectives on Psychological Science.3 (5):400-424. 2008.
- Stoffer, E.S. (2000). The effect of discussion and self-focusing on interpersonal problem solving amongdysphoric and nondysphoric individuals. PhD Thesis, University of Galgary, Canada Sukhodolsky, D. G., Golub, A., & Cromwell, E. N. 2001.

- 19. Watkins, Ed., & Baracaia, S. Why do people ruminate in dysphonic moods? Personality and Individual Difference.30:723-734. 2001.
- 20. Papageorgiou, C., & Wells, A. Positive beliefs about depressive rumination: development and preliminary validation of a self-report scale. Behavior Therapy. 32: 13-26. 2001.
- 21. Nolen_Hoeksema, S. & Davis, C. G.Thank for sharing that: Ruminators and their social support networks. Journal of Psychology. 77:801_814. 1999.
- 22. Fidman, C. (2000) . The treatment social phobia efficacy of cognitive behavior therapy .the british journal of psychology. 2:158-182 . 1996.
- 23. Hudley, C .A. Comorian teacher and Perception of aggression: An ecological approach. Journal of Educational Psychology. 85 (2):377 – 384. (1993).

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The etiology and management of epistaxis amongst Iranian patients: A retrospective study in Boo-Ali Hospital, Sari, Iran

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Abstract

Backgrounds and Objectives: Sixty percent of the world population during their life experience epistaxis, whether spontaneous or secondary to underlying pathology of which 6% of cases require medical management. This study was designed to evaluate and define the probable risk factors and clinical management of epistaxis.

Methods: To retrospectively analyze the clinical records of 100 patients who were treated during one-year from March 2011 to March 2012 at the Department of Otorhinolaryngology, head and neck surgery of Mazandaran University of Medical Sciences Sari, Iran were entered in the study. Descriptive analysis was done to evaluate the results. SPSS software, Version 15, Chicago, IL, USA was used to apply statistical tests. P value of less than 0.05 was defined as significant.

Results: The study involved 47 male and 53 female with mean age of 47 years \pm 21. Hypertension, cardiovascular diseases, diabetes mellitus and hyperlipidemia were observed in 51%, 18 %, 15% and 14% respectively. The commonest way of treatment of bleeding points was anterior tampons (59%) followed by cautery (23%). Ethmoidal and sphenopalatine artery ligation was performed in two and three patients, respectively.

Conclusion: The most common predisposal factor which was noted in this study was hypertension. Clinicians need to control these risk factors before presentation of epistaxis. The routine management of these patients in our center was anterior tampons, cautery and anterior and posterior tampons of the bleeding site.

Keywords: Epistaxis, tampon, risk factor.

Introduction

Sixty percent of people during their life experience epistaxis, whether spontaneous or due to any underlying pathology, of which 6% of these patients needs medical management. [1] The incidence differs with age, the peaks are in children and young adults and the older adult (45-65 years). [2]Although the etiology of epistaxis can be split up to local and systemic factors, the majority of them are idiopathic .In most patients, damage to superficial vessels results in bleeding. Spontaneous rupture of these vessels occurs occasionally, like during extreme valsalva as weightlifting. Neoplasia should be considered when the epistaxis is unilateral and recurrent [3]. There are different options for the management of epistaxis including nasal packing and endoscopic procedures including endoscopic cautery of bleeding points, and more sophisticated methods of endoscopic ligation of the sphenopalatine artery or the anterior ethmoidal artery. Although endoscopic procedures are alternative of nasal packing but former techniques provide economical convenience besides reducing the morbidity and providing convenience to the patient. [4-9] This study was conducted to evaluate the clinical managements, risk factors and etiology of epistaxis in 100 cases which were treated in our center, retrospectively.

Methods and materials

Our retrospective study reviewed 100 patients treated in the Department of Otorhinolaryngology, head and neck surgery of the Mazandaran University of Medical Sciences over 1-year period from March 2011 to March 2012. The ethics committee of the Mazandaran University of Medical Sciences has approved this study. All subjects underwent preoperative routine blood samples. Our inclusion criteria included hemoglobin levels more than 10 mg/dL, normal prothrombin and partial thromboplastine times.

Computed tomography (CT) scan with an axial and coronal projection was applied for patients with a suspected sinus disorders or with evidence of a neoplasia in the nasal cavity. Functional endoscopic sinus surgery (FESS) was performed under local anesthesia (1% lidocaine hydrochloride with 1:100,000 epinephrine). The endoscopic sinus operation procedure has been elucidated in detail by Unlu HH et al (21) was performed using Messerklinger's method and its minor alterations. The surgery finished when there were no points of bleeding and accumulation of blood in nasal cavity or in nasopharynx space. Patients were advised to prevent blowing their nose, or to do unnecessary efforts, which can increase the pressure such as contraction of the abdominal wall, coughing, sneezing. Antibiotic therapy was applied with coamoxiclav (50 mg/kg/8 hours) and tranexamic acid (1 fl twice/day orally) initiating at the night of the operation (10).

Statistical analysis

Descriptive analysis was performed to characterize the outcomes. Data were transferred to MS-excel spread sheets. The procedures involved were transcription, preliminary data inspection, content analysis and at last interpretation. Investigators used percentages (SPSS software, Version 15, Chicago, IL, USA) to interpret epidemiological variables.

Risk factors	number	percent
Hypertension	51	51
Diabetes mellitus	15	15
Hyperlipidemia	14	14
Cardiovascular diseases	18	18
Gastrointestinal disorders	4	4
Thyroid diseases	3	3
Previous epistaxis	3	3
Renal disorders	3	3
Respiratory diseases	2	2
lymphoma	3	3
Thalasemia minor	2	2

Table 1. Risk factors of the study population

The study populations included 47 men (47%) and 53 women (53%) with mean age of 47 years ± 21 . Hypertension (51%) was the most common predisposing factor which was noted in this study. Cardiovascular diseases, diabetes mellitus and hyperlipidemia were observed in 18 %, 15% and 14% respectively (table 1). The commonest way of management of bleeding was anterior tampons (59%) went after by cautery (23%). Ethmoidal and sphenopalatine artery ligation was performed in two and three patients, respectively (table 2).

Discussion

In this study we investigated the predisposal factors and management of epistaxis in 100 cases retrospectively in our center. Our study revealed that hypertension, diabetes mellitus, hyperlipidemia, cardiovascular diseases were the most important risk factors in this series.

The main pathophysiology of epistaxis is not fully understood needing further investigations to clarify the underlying pathology. But injury to superficial blood vessels was subject of researches. [3] Cassano M et al [10] reported probable risk factors in 133 patients with epistaxis. They revealed hypertension in 23 (17.3%) cases, diabetes in 6 (4.5%), asthma in 19 (14.2%), allergies in 36 (27%) and other pathologies (nephropathologies, hepatitis and etc.) in 5 (3.7%). But in this investigation we observed hypertension in 51 subjects, diabetes mellitus in 15 patients went after by other pathologies (table 1).

The traditional control of epistaxis depends on diagnosis of the bleeding site by using a head mirror or light source. Upon local determination

 Table 2. Clinical management of the series

Procedures	Number	Percent	
Ethmoidal Artery Ligation	2	2	
Sphenopalatine Artery	2	2	
Ligation	3	3	
Anterior Tampons	59	59	
Posterior Tampons	4	4	
Anterior And Posterior	0	0	
Tampons	9	9	
Cautery	23	23	

of a bleeding point chemical or electrocautery is performed. If these procedures didn't respond then anterior packing or more advanced methods like compressive balloons, posterior packing or arterial ligation or embolisations are prescribed (3). In the current study anterior tampons applied for 59 patients, cautery in 23 cases, anterior and posterior tampons in 9 subjects, posterior tampons in 4 participants, sphenopalatine artery ligation in 3 cases and ethmoidal artery ligation in 2 patients (table2). These findings indicated anterior tampons and cautery of the bleeding points were the commonest way of management in this study. Michele Cassano et al (10) studied 130 cases of epistaxis retrospectively. They indicated Only 16 patients (12%) underwent nasal packing, while in the remaining 117 (88%) endoscopic control of bleeding allowed avoiding packing. In 53 (39.8%) cases an endoscopic cauterization of bleeding points was applied; in 29 (21.8%) subjects a sphenopalatine artery ligation was performed. in their investigation 2 subjects (1.5%) underwent anterior ethmoidal artery ligation. In the left 34 (25.5%) participants no procedure was necessary, due to scarce bleeding in the endoscopic vision at the end of operation. In this group of non-packed subjects, only 8 (6.8%) required a post-operative tamponade while in the group of packed patients, 2 (12.5%) patients had rebleeding and revisional surgery was crucial. In contrast with the current study most of the patients in Cassano study (88%) received endoscopic management of the bleeding point whereas in our research 59% of the patients underwent anterior tampons.

Posterior ethmoidal artery ligation is not routinely applied due to risk of blindness (the artery is very close to the optic nerve) and since it usually does not result in severe bleeding (11). Based on Bugten study, performing a Merocell packing in the medial meatus is useful to avoid not only epistaxis but also synechiae of the middle turbinate with the lateral wall (12). The management of synechiae between the middle turbinate and nasal septum prevent the requirement to pack the nose to bolster the mucosa or to medialize the middle turbinate. Merocel is made of polyvinyl alcohol, a compressed foam polymer which is located into the nose and expanded by application of water. This imposes the tampon to swell and fill the nasal cavity, applying pressure over the bleeding site. It might allow clotting factors to localize and reach a critical state, so will make the coagulation easier. Merocels are easy to put into a casualty setting and need a little training. They are effective and useful in 85% of the patients, without any changes between the success rates when are compared with traditional ribbon gauze (13).

Conclusion

The most common predisposal factors which were noted in this study were hypertension followed by cardiovascular diseases, diabetes mellitus and hyperlipidemia. The routine management of these patients was anterior tampons, cautery and anterior and posterior tampons.

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References

- 1. Petruson B, Rudin R. The frequency of epistaxis in a male population sample. Rhinology 1975;13:129– 133.
- 2. Watkinson JC. Epistaxis. In: Mackay IS, Bull TR, eds. Scott Brown's otolaryngology. London: Butterworths, 1997;18/5–7.
- 3. Pope L, Hobbs C. Epistaxis: an update on current management. Postgrad Med J 2005 81: 309-314.
- 4. Trinidad Ruiz G, Rejas Ugena E, Gonzale's Palomino A, Pantoja Hernandez CG, Mora Santos ME, Blasco Huelva A. Practical aspects regarding endoscopic treatment for epistaxis. Acta Otorrinolaringol Esp 2006;57(9):394–400.
- 5. Rejas Ugena E, Trinidad Ruiz G, Alvarez Dominguez J, Carrasco Claver F, Pino Rivero V, Blasco Huelva A. Utility of surgical treatment for severe epistaxis by endoscopic approach of sphenopalatine and ethmoidal arteries. Acta Otorrinolaringol Esp 2006;57(5): 228–234.
- 6. Umapathy N, Quadri A, Skinner DW. Persistent epistaxis: what is the best practice? Rhinology 2005;43(4):305–308.

- 7. Kumar S, Shetty A, Rockey J, Nilssen E. Contemporary surgical treatment of epistaxis. What is the evidence for sphenopalatine artery ligation? Clin Otolaryngol 2003;28:360–363.
- 8. Voegels RL, Thome' DC, Iturralde PP, Butugan O. Endoscopic ligature of the sphenopalatine artery for severe epistaxis. Otolaryngol Head Neck Surg 2001;124(4):464–467.
- 9. Wormland PJ, Wee DT, Van Hasselt CA. Endoscopic ligation of the sphenopalatine artery for refractory posterior epistaxis. Am J Rhinol 2000;14(4):261–264.
- Cassano M, Longo M, Fiocca-Matthews E, Maselli Del, Giudice A.Endoscopic intraoperative control of epistaxis in nasal surgery. Auris Nasus Larynx 2010; 37: 178–184
- 11. Kumar S, Shetty A, Rockey J, Nilssen E. Contemporary surgical treatment of epistaxis. What is the evidence for sphenopalatine artery ligation? Clin Otolaryngol 2003;28:360–3.
- 12. Bugten V, Nordgard S, Skogvoll E, Steinsvag S. Effects of nonabsorbable packingin middle meatus after sinus surgery. Laryngoscope 2006;116(1):83– 8.
- 13. Corbridge RJ, Djazaeri B, Hellier WP, et al. A prospective randomized controlled trial comparing the use of merocel nasal tampons and BIPP in the control of acute epistaxis. Clin Otolaryngol 1995;20:305–7.

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Dose distribution determination of ⁹⁰Sr/⁹⁰Y beta brachytherapy source using Monte Carlo code

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Abstract

Introduction: A ⁹⁰Sr/⁹⁰Y source with 12-seed source train has been considered in order to obtain dosimetery parameters. This beta emitter source is used in radiation therapy of coronary artery to prevent restenosis.

Materials and Methods: The Monte Carlo MCNPX code is used to determine the percent depth dose, reference dose rate and two dimensional dose rate distributions. Then two dimensional of dose rate is tabulated. The coefficient of variation of dose rate is determined in parallel direction with long source axis for various depths to determine the uniformity of data. The dose rate of different source is also drawn to compare the results and validate them.

Result and Discussion: The study results show the smooth percent depths dose profile with rapid dose fall off. The reference dose rate is calculated to be 5.786 cGy $h^{-1} \mu Ci^{-1}$. The coefficient of variation of dose rate is increased in depth. The dose rate is also validated with the previous publication.

Conclusion: Due to short ranges of beta particles, the dose profile of beta emitter source fall off rapidly. Beta particles expose to treatment region with acceptable dose rate. Short ranges of beta particles cause the negligible damage to healthy adjacent organ.

Keywords: Dose, Beta Brachytherapy, ⁹⁰Sr/9⁰Y, treatment of restenosis, MCNPX

Introduction

Cardiac disease is one of the most important causes of death in the world. Coronary artery stenosis is a very common cardiac disease. Intravascular brachytherapy (IVBT) is one of the radiotherapy methods which have been used recently in coronary artery radiation therapy for the treatment of restenosis (Saghamanesh et al., 2011).

The technique of irradiating the vessel internally by short-range ionizing radiation is called intravascular brachytherapy (IVBT), and, in general, the isotopes of choice for IVBT are beta emitters. These isotopes are used because they cause insignificant irradiation to adjacent organs and also, the personnel in the catheterization laboratory are subjected to insignificant exposure during treatment time. Different radiation sources (⁹⁰Sr/⁹⁰Y, ³²P, ¹⁸⁸W/¹⁸⁸Re) have been available commercially to be used for these treatments. The use of ionizing radiation requires a precise knowledge of the absorbed dose delivered during the treatment to administer the dose locally, where is needed, by avoiding any damage to the surrounding healthy tissue (Demir et al., 2009; Amols, 1999). Dosimetric studies have been reported for various beta sources. Saghamanesh et al. (2011) used a Monte Carlo simulation to calculate the dosimetry parameters and effective equivalent doses to the heart and its surrounding tissues during IVBT. They also calculated the reference absorbed dose rate and reported to be 1.097 cGy min⁻¹ mCi⁻¹ which is equivalent with 6.582 cGy h⁻¹ µCi⁻¹. Taghdiri et al. (2011) calculated the dosimetric parameter of AAPM TG-60 using MCNP4C code data. They reported the dose rate value at the reference point for ¹⁵³Sm sources to be 9.41cGy h-1 µCi-1. Jung and Reece (2008) calculated the dose distribution using MCNP5 and measured using radio chromic film. They reported the dose rate value at the reference point for ¹⁴²Pr to be 2.412 cGy $h^{-1}\mu$ Ci⁻¹. Mourtada et al. (2004) used Monte Carlo simulations MCNPX to determine the AAPM Task Group 60 parameter for ³²P source. They reported the dose rate value at the reference point to be 1.081 cGy h⁻¹ μ Ci⁻¹. Wang and Li (2002) reported the EGSnrc Monte Carlo results of two-dimensional dose rate distributions in water and also calculated the TG-60 dose parameters. They reported the dose rate value at the reference point for ⁹⁰Sr/⁹⁰Y sources to be 6.0 cGy h⁻¹ μ Ci⁻¹.

The Monte Carlo code MCNPX has been used to simulate the transport of electrons emitted by the encapsulated ⁹⁰Sr/⁹⁰Y source to calculate the dosimetry parameters.

In this study, a 90 Sr/ 90 Y source has been considered in order to obtain dosimetriy characteristic. The objective of this study is to measure the dose rate of 90 Sr/ 90 Y IVBT sources and the dose uniformities of these sources along the source axis. The reference dose rate is defined at the source bisector orthogonal to source long axis at a radial distance of 2 mm and θ =90°. The percent depth dose which normalized to reference dose has been calculated. The coefficient of variation of the dose rate has been also calculated. The dose rate of different sources has been also drawn.

Materials and Methods

90Sr/90Y source and phantom

Sr-90 is abundant long-lived fission product and decays with half-life of 28.90 years into shortlived daughter Y-90, which in turn decays with half-life of 64 hours into stable Zr-90. The ⁹⁰Sr/⁹⁰Y source is a pure beta-emitting radionuclide which the maximum beta energy from the Sr-90 (Emax) is 0.546 MeV and with mean kinetic energy of 0.196 MeV, while Y-90 produces more energetic and penetrating beta particles with maximum energy (Emax) of 2.284 MeV and mean kinetic energy of 0.933MeV. Since the daughter, Y-90 has such a short half-life, its emission is in secular equilibrium with its parent, Sr-90 (Demir et al., 2009).

The 90 Sr/ 90 Y source, supplied by Novoste consists of 12 seeds. Each seed has a ceramic cylinder core encapsulated by a SS304 stainless steel capsule. The composition of the ceramic core assumed to be SiO₂. The diameter and height of the core

are 0.56 and 2.5 mm, respectively. The thickness of the steel capsule is taken as 0.04 mm on the side and 0.05 mm on the top and the bottom of the cylindrical core. A 2.54-mm long proximal/distal gold marker attached to each end of the source train (Demir et al., 2009; Li et al., 2001; Soares et al., 1998; Wang and Li, 2000; Ye et al., 2000).

In this study, the calculation of dose distribution is done around the source of 90 Sr/ 90 Y placed in the center of a cubic water phantom with dimensions 30*30*30 cm³ with the Monte Carlo code MCNPX. The source long axis is on Z axis and the dosimetry calculation is in YZ plane.

The dose calculation in Y and Z direction is done in spheres with radius of 0.2mm and distances of 0.5mm. The *F8 tally is used to score deposited energy (MeV) in the detectors around the source, and it is divided by the detectors mass, and then multiplied by appropriate conversion factor to obtain absorbed dose. In this research electron energy cut off is 1KeV and the relative error is below 2%. The energy spectra of ⁹⁰Sr/⁹⁰Y source were used as input in the simulations is shown in Figure 1 (Li et al., 2001; Cross et al., 1983).

Monte Carlo MCNP code

Monte Carlo MCNP code appears to be a strong candidate compared to Electron Gamma Shower (EGS), especially when the powerful geometry capabilities of MCNP are taken into account (Ljungberg, 1998).

Due to the rapid dose decrease around the beta sources, it is extremely difficult to obtain accurate dose experimentally. Therefore, Monte Carlo calculation is widely used as an alternative method to analyze the dose distribution around the radioactive source for therapeutic purposes. In this study



Figure 1. The beta energy spectrum of 90Sr/90Y source

MCNPX code is used to calculate the dosimetric parameters of the ⁹⁰Sr/⁹⁰Y source.

Beta-emitting radionuclides predominantly interact with atoms in a medium by Coulomb interactions. Inelastic collisions with atomic electrons lead to energy loss by ionization and excitations, and inelastic collisions with nuclei lead to bremsstrahlung production. Elastic collisions with atomic electrons and nuclei, however, do not result in energy deposition. Energy deposition occurs predominantly through ionizing events with atomic electrons in low atomic number media (e.g., water), with bremsstrahlung being more prevalent with higher atomic number materials. These inelastic modes of energy loss through a medium are calculated here using the Monte Carlo simulation technique. This method provides a solution to the electron transport equation implicitly by simulating many electron trajectories and their secondary by using random sampling techniques. Monte Carlo results are highly statistical in nature, but using a large number of electron histories can mitigate this problem. The Monte Carlo method is an excellent dosimetric tool in terms of accuracy when the source geometry is well modeled (Klevenhagen, 1993).

Beta dosimetry

Beta dosimetry refers to the techniques which measures the β -dose component of the radiation. The Monte Carlo N-Particle (MCNP) code will be used in this study to simulate the set up and to determine the dose distribution as radiation passes from the source to the water phantom and also the dose rates at various depths in an object (water phantom).

In such decay sequence the nuclide which decays is frequently called the parent and its decay product the daughter. If both the parent and daughter nuclides are radioactive and the parent has longer half-life than the daughter, the rate of decay of the daughter is determined not only by its own half-life but also by the rate at which it is produced. As a first approximation assume that if the activity of the parent remains constant, or is constantly replenished, so that the rate of production will at first exceed its rate of decay and equilibrium will be reached when rate of production is just equal to the rate of decay (Dendy and Heaton, 1999). The rate of approach to equilibrium depends on half-life of the daughter, for example ⁹⁰Sr/⁹⁰Y, after 10 half-lives, the activity will be within 0.1% of equilibrium. The equilibrium activity is governed by the activity of the parent, Sr-90 (Dendy and Heaton, 1999; Cherry et al., 2003; James, 2006).

Depth dose distribution is defined as the measure of energy variation with depth and deposited in a water phantom. Lower readings can be observed with increasing depth owing to the attenuation of radiation by the water. The dose is calculated by tallying the energy deposited in each cell of the phantom. The energies were represented by *F8: pe, giving the deposited energy in the tally cell in MeV. Thus, for each photon and electron entering the tally cell, the total energy deposited in that cell is calculated from the difference between the energy carried into (entered) and energy carried out of the cell by the particles. This is also known as the absorbed dose tally and scores energy deposited only if there is a photon interaction or electron interaction within the cell, or when an electron crosses into the tally cell. In the case of both particles, if there is no interaction the net energy absorbed is zero. The absorbed dose is obtained by dividing the *F8 number by the mass of the cell. For example, in this simulation cell 10 received energy of 4.54344*10⁻⁵ MeV per source particle. By dividing the net energy deposited in the cell by the mass of a cell, an average dose in cell is obtained. Dose in cell 10 which is in water phantom is calculated as follows: Dose (cell 10) = dE/dm. Cell 10 is a sphere with radius of 0.2mm and the density of 1000kg/m³. The mass of cell 10 is 33.51*10⁻⁹ kg. In order to calculate the dose rate, the activity is required. Since the simulations were run with Sr-90 + Y-90 which give out two particles per disintegration, then the dose rate is obtained in cGy $h^{-1}\mu Ci^{-1}$ with the following equation $\dot{D}(cGy,h^{-1},\mu CI^{-1}) = R(MeV) \frac{2\left(\frac{particle}{Bq}\right) \times 1.602 * 10^{-13} \left(\frac{j}{MeV}\right) \times 3.7 * 10^{4} \left(\frac{Bq}{\mu CI}\right) \times 100 \left(\frac{cGy}{Gy}\right) \times 3600 \left(\frac{sec_{h}}{h}\right)}{m(kg)}$

Result and Discussion

Percent depth dose

Figure 2 shows percent depth dose for Z=5, 10, 15, 20 mm in different Y. The doses were normalized to the dose at Z=2mm on the central plane bisecting the source. This Figureure shows with

the increment of Y, the dose profile quickly reduced and drop. The dose fall off rates are related to the beta penetration in water and so the short range of beta particle. Figure 3 shows the percent depth dose profile in parallel direction with source axis for different depth, Y=1, 2, 3, 4, 5, 6 mm. This Figureure also shows rapid dose fall off of beta particle. The dose profile across the source length for different depth is almost the same and out of this distance reduces rapidly.



Figure 2. Percent depth dose for various depths along different Z

Dose rate

The dose rate is presented in table 1 in Cartesian coordinate in Y and Z. The data are recorded from 0 to 20 mm in z direction in 0.5mm step distance and from 0.58 to 6 mm in Y direction. According to table data, the dose rate reduces exponentially in Y direction with distance from the source. Variance and standard deviation of data at different distances of source are useful parameter of data



Figure 3. Percent depth dose for various depths parallel to source

Table 1. Tow dimension distribution of dose rate for the ${}^{90}Sr/{}^{90}Y$ *source (cGy h*⁻¹ μ *Ci*⁻¹)

z(mm)	y=0.58mm	y=1mm	y=1.5mm	y=2mm	y=2.5mm	y=3mm
0	30.60029	15.359	8.534522	5.78639	3.978278	2.7043
0.5	31.06502	15.6566	8.509411	5.89702	4.39195	2.73149
1	31.28325	15.6183	8.499531	5.9698	4.449007	2.62954
1.5	31.65066	15.4171	8.682289	5.43572	4.155916	2.63861
2	31.38736	14.9918	8.794308	5.3349	4.189151	2.66797
2.5	31.32272	15.9076	8.537471	5.61002	4.049274	2.8217
3	32.0747	16.1054	9.087882	5.85028	4.070282	2.76789
3.5	32.14201	15.962	9.479651	6.08871	3.951162	2.80943
4	32.77167	16.1826	9.145651	5.87591	4.241639	2.90839
4.5	32.30221	15.9674	9.321053	6.25065	4.248066	3.0162
5	31.9498	16.4086	9.48549	6.33806	4.002699	2.96608
5.5	32.28264	16.5182	9.485668	6.08666	4.270109	3.29263
6	32.26584	16.7276	9.870394	6.11995	4.326567	3.02774
6.5	32.94257	16.2348	9.452282	6.4942	4.223818	3.06219
7	32.43665	16.3751	9.33398	6.24655	4.474738	2.97982
7.5	31.84733	16.5498	9.307458	6.08625	4.250799	2.93695
8	32.35218	16.2287	9.425581	6.10141	4.127928	2.99871
8.5	32.45965	16.4684	9.77224	6.45493	4.285237	2.97042
9	33.08085	16.6655	9.597328	6.38009	4.123814	3.13156
9.5	32.85004	16.247	9.639318	6.43831	4.27807	3.05181
10	32.22987	16.2897	9.937747	6.53196	4.605967	3.05132
z(mm)	y=3.5mm	y=4mm	y=4.5mm	y=5mm	y=5.5mm	y=6mm
0	1.814	1.093	0.659	0.623	0.383	0.22
0.5	1.833	1.119	0.738	0.66	0.384	0.219

1	1.867	1.124	0.754	0.657	0.375	0.224
1.5	1.881	1.141	0.756	0.677	0.373	0.222
2	1.903	1.235	0.769	0.68	0.381	0.231
2.5	1.921	1.184	0.776	0.684	0.392	0.215
3	2.116	1.195	0.887	0.651	0.418	0.274
3.5	2.119	1.399	0.82	0.67	0.442	0.261
4	2.119	1.329	0.834	0.679	0.443	0.29
4.5	2.129	1.51	1.097	0.645	0.395	0.245
5	2.122	1.44	1.009	0.739	0.393	0.265
5.5	1.991	1.579	1.052	0.661	0.418	0.339
6	1.995	1.48	1.088	0.714	0.409	0.258
6.5	2.189	1.376	0.95	0.702	0.477	0.247
7	2.024	1.532	1.013	0.694	0.398	0.261
7.5	2.128	1.501	1.015	0.632	0.415	0.334
8	1.902	1.472	1.039	0.62	0.433	0.254
8.5	2.223	1.405	0.942	0.555	0.505	0.276
9	2.117	1.569	0.947	0.729	0.436	0.274
9.5	2.187	1.359	0.906	0.624	0.419	0.239
10	2.178	1.378	0.973	0.67	0.454	0.347
z(mm)	v=0.58mm	v=1mm	v=1.5mm	v=2mm	v=2.5mm	v=3mm
10.5	31.98649	16.2676	9.651888	6.39462	4.316631	2.91734
11	32.95777	16.3214	9.513617	6.09833	4.402755	3.04365
11.5	32.62908	15.8422	9.602187	6.21833	4.240052	3.03297
12	32.8232	16.5479	9.544819	6.26094	4.152074	2.91079
12.5	31.67192	16.4263	9.225707	6.16275	4.185051	2.83399
13	32.37937	16.3209	9.261234	6.18789	4.039812	2.78497
13.5	32.34224	15.9826	9.282184	5.91897	4.066548	2.82887
14	32.47552	15.203	8.778666	5.64434	3.71886	2.34892
14.5	30.39704	14.8928	8.073714	5.44785	3.38823	2.11096
15	22.98214	11.5857	7.085266	4.31511	2.977745	2.06166
15.5	6.991615	6.37719	4.720257	3.36462	2.25996	1.7252
16	2.890797	3.53143	2.863874	2.4132	1.817318	1.29185
16.5	1.535712	1.81784	1.825203	1.61738	1.257413	1.03125
17	0.983808	1.19023	1.197277	1.05906	0.990289	0.70107
17.5	0.771141	0.76689	0.763777	0.72991	0.644834	0.4759
18	0.546787	0.51608	0.451838	0.47146	0.448905	0.33614
18.5	0.28524	0.29404	0.313392	0.26447	0.292423	0.1844
19	0.221001	0.26109	0.20786	0.20288	0.154467	0.13807
19.5	0.165372	0.17718	0.160902	0.11617	0.111623	0.06282
20	0.064292	0.04862	0.094924	0.06187	0.095431	0.04298
z(mm)	y=3.5mm	y=4mm	y=4.5mm	y=5mm	y=5.5mm	y=6mm
10.5	2.102383	1.4969	1.065415	0.67039	0.482617	0.2457
11	2.109992	1.54293	1.085403	0.78418	0.478734	0.26993
11.5	2.007492	1.43519	0.94221	0.68335	0.409581	0.28979
12	2.119651	1.4831	1.016454	0.59996	0.509772	0.27137
12.5	1.930489	1.4419	0.989246	0.64203	0.310997	0.28327
13	1.902665	1.33889	0.857304	0.64794	0.395389	0.25972
13.5	1.972265	1.3766	0.933153	0.62824	0.438853	0.20744

14	1.772279	1.15191	0.86362	0.5177	0.265142	0.20365
14.5	1.622147	1.14889	0.720027	0.55805	0.293552	0.19428
15	1.251805	0.99767	0.718449	0.39017	0.240463	0.13348
15.5	1.186967	0.94929	0.540203	0.39116	0.180706	0.15622
16	0.9347	0.66702	0.530735	0.34348	0.180151	0.11195
16.5	0.694548	0.58198	0.352505	0.2589	0.166301	0.09534
17	0.541509	0.41682	0.275139	0.16323	0.124727	0.08764
17.5	0.376593	0.29627	0.200205	0.14527	0.086735	0.05883
18	0.308579	0.19091	0.13665	0.09831	0.078236	0.0471
18.5	0.163502	0.14408	0.08879	0.07689	0.082225	0.02882
19	0.138513	0.09384	0.075647	0.04684	0.033904	0.0167
19.5	0.036663	0.05088	0.035926	0.01924	0.006063	0.01055
20	0.046451	0.02257	0.017635	0.0261	0.010246	0.01594

table to check the uniformity of data in parallel direction of source. But the dependence of these parameters to the magnitude of data leads not to be the determinant of the data uniformity.

Therefore, to compare the results, the coefficient of variation is used. The coefficient of variation is obtained by dividing the standard deviation with the average value. The coefficient of variation is a dimensionless and normalized parameter and is not dependent to the magnitude of data.

In Figure 4 the coefficient of variation is drawn in parallel direction with source for various distances from source. This coefficient is increased with the distance from the source and implies on increment of data scattering. Two reasons can be cited for increment of data scattering.

The first reason is the statistical variations which increase because of reduction beta particles number far from the source and therefore coefficient of variation increase.

The second reason of the increment of coefficient of variation for a line source with uniform distribution is the variation in emitted beta particle energy. Firstly, beta particles energy is continues





spectrum so beta particle range is different and the beta particles number in the same depth along the source length is different. Secondly, different beta particles with the same energy deposit different energy in their trajectory, so they have a different range.

Of course, the small coefficient of variation at near depth is determination of the dose uniformity along the source length.

Figure 5 shows the comparison of dose rate profile of various sources (Mourtada et al., 2004; Asenjo et al., 2009). The dose rate is in cGy h⁻¹ μ Ci⁻¹. The maximum energy of ¹⁴²Pr beta is 2.162 MeV and maximum beta energy of ⁹⁰Sr/⁹⁰Y and 32P are 2.282 MeV and 1.709 MeV, respectively. The ¹⁴²Pr decays by beta emission at 96.6% of the time and is followed by emission of 1.575 MeV gamma ray with 3.7%. ³²P decay to ³²S by beta emission with 100% abundance. This Figureure shows the higher dose rate of ⁹⁰Sr/⁹⁰Y and also that the dose rate reduce exponentially.

The dose profile of γ decreased gradually, but the dose profile from β - has dropped sharply. The dose fall off rates are related to the mean (or maxi-



Figure 5. Comparison of dose rate of various beta emitters

mum) beta energies. As with gamma-emitting sources, beta-emitters showed very high dose rate gradients at close distances to the source, but unlike gamma emitters, insignificant doses at larger distances.

Conclusion

Dosimetric parameter, including percent depth dose, reference dose rate and dose rate of 90 Sr/ 90 Y beta brachytherapy source is determined with Monte Carlo MCNPX code. This calculation compared with the previous published data. There is a good agreement between them. The reference dose rate is calculated to be 5.786 cGy h⁻¹ µCi⁻¹. The dose rate data is tabulated and can be used for treatment planning for intravascular brachytherapy. Due to short ranges of beta particles, the dose profile of beta emitter source fall off rapidly. Beta particles expose to treatment region with acceptable dose rate. Short ranges of beta particles cause the negligible damage to healthy adjacent organ.

Reference

- 1. Amols, HI., 1999. Review of endovascular brachytherapy physics for prevention of restenosis. Cardiovasc Radiat. Med., 1, 64-71.
- Asenjo, J., Fernandez, Varea, JM., Sanchez, Reyes, A., 2002. Characterization of a high dose rate 90Sr/90Y source for intravascular brachytherapy by using the Monte Carlo code PENELOPE. Phys. Med. Biol., 47(5), 697-711.
- 3. Cherry, S.R., Sorenson, J.A., Phelps M. E., 2003. Physics in Nuclear Medicine. 3rd edition, Philadelphia U.S.A, Saunders, ISBN: 0-7216-8341-X.
- 4. Cross, WG., Ing, H., Freedman, N., 1983. A short atlas of beta-ray spectra. Phys. Med. Biol., 28, 1251–1260.
- Demir, B., Ahmed, A. S., Babalik, E., Demir, M., Gurmen, T., 2009. Verification and uniformity control of doses for 90Sr/90Y intravascular brachytherapy sources using radiochromic film dosimetry. Journal of Medical Physics, vol. 33, P. 54-59.
- 6. Dendy, P.P., Heaton, B., 1999. Physics for Diagnostic Radiology. 2nd edition. Bristol Philadelphia: U.S.A, Institute of Physics (IOP) Publishing. ISBN: 0-7503-0591-6.
- James, E. M., 2006. Physics for Radiation Protection. 2nd edition. Germany, Weinheim Wiley-VCH, ISBN: 3-527-40611-5.

- 8. Jung, JW., Reece, WD., 2008. Dosimetric characterization of 142Pr glass seeds for brachytherapy. Applied Radiation and Isotopes. 66(4), 441-9.
- 9. Klevenhagen, S.C., 1993. Physics and dosimetry of therapy electron beams. Madison Wisconsin, Medical Physics (IOP) Publisher, ISBN: 0-944838-36-7.
- 10. Li, X.A., Suntharalingam, M., Cedric, Yu., 2001. Dosimetry of source stepping for intravascular brachytherapy. Cardiovascular Radiation Medicine. 2, 65–172
- Ljungberg, M., Strand, S. E., King, M.A., 1998. Monte Carlo Calculations in Nuclear Medicine: Applications in Diagnostic Image. Bristol Philadelphia: U.S.A, Institute of Physics (IOP), ISBN: 0-7503—0479-0.
- 12. Mourtada, F., Soares, CG., Horton, JL., 2004. A segmented 32P source Monte Carlo model to derive AAPM TG-60 dosimetric parameters used for intravascular brachytherapy. Medical Physics. 31: 602-608.
- 13. Soares, CG., Halpern, DG., Wang CK., 1998. Calibration and characterization of beta-particle sources for intravascular brachytherapy. Med. Phys. 25, 339–346.
- 14. Saghamanesh, S., Karimian, A., Abdi, A., 2011. Absorbed dose assessment of cardiac and other tissues around the cardiovascular system in brachytherapy with 90Sr/90Y source by Monte Carlo simulation. Radiation Protection Dosimetry. pp. 1–4.
- 15. Taghdiri, F., Sadeghi, M., Hosseini, S.H., Athari, M., 2011. TG-60 dosimetry parameters calculation for the beta-emitter 153Sm brachytherapy source using MCNP. Iran. J. Radiat. Res., 9 (2), 103-108.
- 16. Wang, R., Li, X.A., 2000. Monte Carlo calculation of a Sr/Y-90 and an Ir-192 sources for intravascular brachytherapy. Med. Phys., 27, 2528–2535.
- 17. Wang, R., Li, X.A., 2002. Dosimetric comparison of two 90Sr/90Y sources for intravascular brachytherapy: an EGSnrc Monte Carlo calculation. Phys. Med. Biol. 47, 4259-4269.
- Ye, SJ., Li, X.A., Zimmer, JR., 2000. Dosimetric perturbations of linear array of beta emitters and metallic stents in intravascular brachytherapy. Med. Phys. 27, 374–380.

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The occurrence of dentine hypersensitivity after ultrasonic scaling in patients with periodontitis

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Abstract

Ultrasonic scaling is a basic procedure for removal of dental deposits applied in order to limit the action of the bacterial factor in treatment of periodontitis. However, the application of ultrasonic scalers has certain disadvantages. According to literature, it may lead to post-treatment dentine hypersensitivity, which causes discomfort in patients and hinders the correct performance of hygienic procedures at home. The aim of the study was to assess the sensitivity of teeth before, directly after and two weeks after ultrasonic scaling in patients with chronic periodontitis. The study included 300 teeth. Dentine sensitivity to the stimulus in the form of an air-jet from a blower was examined three times, assessing the sensitivity of selected teeth using the 4-grade Schiff Sensitivity Score. Dentine sensitivity before scaling was observed in 34.6% of studied teeth and in 47.6% of recessed teeth. Directly after scaling and polishing, the percentage of sensitive teeth fell down to 30.2% for all teeth, and down to 29.8% in the recessed teeth group (p=0.006), but then after two weeks it rose to 41.5%, exceeding the initial values (p=0.019). Ultrasonic scaling may cause dentine sensitivity. Thorough polishing may limit the occurrence of hypersensitivity directly after the scaling procedure.

Key words: Dentine hypersensitivity, ultrasonic scaling.

Introduction

The initial etiological factor for chronic periodontitis are bacteria present in dental plaque. Therefore successful treatment of the disease highly depends on proper removal of dental deposits. Initially it should be performed in the dental surgery by a qualified personnel – a dentist or a dental hygienist. After receiving training on oral hygiene, including correct methods of brushing and application of proper brushes and other equipment used for cleaning interdental spaces, the patient should maintain a good level of domestic hygiene, which constitutes a vital supplementary and supporting element of therapy.

Correct performance of professional calculus removal depends on many factors, thus it carries a risk of certain undesirable effects.

According to literature, one of the complications of scaling may be iatrogenic damage of tooth root surface (1,2). This results in opening of numerous dentinal canals, which on the other end are open to dental pulp. According to the hydrodynamic theory (3), the liquid flow in dentinal canals, which is stimulated by external thermal, chemical or mechanical stimuli, may cause pain due to irritation of odontoblast endings. This pain is called dentine hypersensitivity.

Scaling, i.e. professional removal of all – both soft and hard – subgingival and supragingival dental deposits can be performed with the use of hand or machine tools. Nowadays hand scaling is effected mostly with universal and special curette, whose working surface is compatible with the root or crown's surface being cleaned. Hand tool treatment requires good qualifications from the operator: knowledge of tooth and the surrounding tissue anatomy, as well as considerable experience and skillfulness.

It should be pointed out that exerting too much force and application of curette surface which is not compatible with the surface of the root may cause damage to root cement and root dentine, which can lead to root decay and dentine hypersensitivity. Moreover, precise work with hand tools is timeconsuming and requires much physical effort – the operator's wrists are particularly vulnerable.

Because of the above mentioned factors, currently professional removal of dental deposits is conducted with the use of special devices - sonic / air scalers or ultrasonic scalers. The operating frequency of sonic scalers is about 6 kHz, with a circularly vibrating tip. Ultrasonic scalers operate with the frequency of 15-50 kHz. They may have a piezoelectric or a magnetostrictive mechanism. Magnetostrivtive scalers the tip is vibrating in an ellipsoid manner. These devices generate a lot of heat, therefore they require adequate cooling. The tip of piezoelectric scalers vibrates linearly. While working with any type of mechanical scalers, one should remember about correct positioning of the scaling tip with reference to the surface of the processed tooth - parallel or with aberration of maximum 15°. A higher aberration or work with perpendicularly positioned tip may cause damage in enamel surface or in dentine.

As it was mentioned before, the application of machine scalers has certain disadvantages. One of the basic faults is the occurrence of dentine hypersensivity both during and after scaling. Parts of teeth not covered by enamel or cement are particularly susceptible. The cause for this may be cavities of non-carious origin, gingival recession, and root exposure due to periodontal tissue loss resulting from the course of periodontitis.

The aim of this study was to assess the sensitivity of teeth before, directly after and two weeks after ultrasonic scaling in patients with chronic periodontitis.

Material and Methods

The study included 300 teeth in patients with chronic periodontitis, who were scheduled for ultrasonic scaling. Only teeth with remaining live pulp, not covered with prosthetic crowns were qualified for the study. Gingival recession was taken into account during the clinical examination. Dentine sensitivity was examined three times: before scaling, immediately after ultrasonic scaling and two weeks after ultrasonic scaling. The reaction to a dehydrating stimulus (cold air and intense drying of dentine surface) was assessed after air jet from a dental blower was pointed towards the examined surface in the vicinity of tooth neck from the distance of 0.5 cm for three seconds or until a demand for termination of the stimulus action. The sensitivity of the examined tooth was assessed using the 4-grade Schiff Sensitivity Score, where 0 - no subject response to stimulus; 1 - subject responds to stimulus; 2 - subject responds and requests discontinuation of stimulus; 3 - subject responds to stimulus and requests discontinuation due to explicit pain. (4). Scaling was performed with the use of a piezoelectric scaler EMS mini piezo, whose power was set in the middle of the scale, with the vibrations frequency range from 24 to 32 kHz. After scaling, polishing was performed with the use of soft rubbers and brushes on lowspeed prophylaxis handpiece, with polishing paste made ex tempore from pumice and glycerin mixed together into rare paste consistence. Each patient was instructed with regard to oral hygiene, including the application of non-traumatic tooth sweeping technique with a soft brush.

Results

The tables show collective results for teeth reacting and not reacting to stimuli in three consecutive examinations. Dentine sensitivity to an air jet from a blower was initially observed in 34.6% of studied teeth (Table 1). In the teeth group with gingival recession (n=84) the percentage was higher and equaled 47.6% (Table 2). After scaling with root surface polishing the percentage slightly decreased for all teeth to 30.2% but the difference was not statistically significant (p=0.16). On the other hand, the percentage of sensitive teeth with recession directly after scaling fell significantly (p=0.006) to 29.8%. During the next examination after two weeks, the percentage of sensitive teeth increased in both groups to 41.5% (Table 1) and 59.5% (Table 2) respectively, exceeding the initial values. However only in the group of all teeth the difference was statistically significant (p=0.019).

The assessment of differences in the percentage of teeth reacting and teeth not reacting to stimuli before and after treatment was performed with the use of the McNemar test.

Apart from that, the relative risk (RR) was evaluated, denoting how many times higher risk of reaction lies with a tooth which reacted also before treatment. The risk of reaction in the second examination for teeth demonstrating sensitivity

			Respo	onse to llus (2)	Total
			No	Yes	
Response to stimulus (1)	No	N	167	30	197
	INO	%	84.8%	15.2%	65.4%
	V	N	43	61	104
	res	%	41.3%	58.7%	34.6%
Total		N	210	91	301
		%	69.8%	30.2%	100.0%

Table 1. Assessment of percentage differences in teeth reacting and not reacting to an air jet from a blower before (1), directly after (2), and two weeks after scaling (3)

 $p_{McNemar} = 0.160$ RR=3.852 (2.669 - 5.558)

			Respo stimu	onse to Ilus (3)	Total	
			No	Yes		
	No	Ν	150	47	197	
Response to	INO	%	76.1%	23.9%	65.4%	
stimulus (1)	Yes	N	26	78	104	
		%	25.0%	75.0%	34.6%	
Total		N	176	125	301	
		%	58.5%	41.5%	100.0%	

 $p_{McNemar} = 0.019$

 $RR=3.144 \ (2.393-4.130)$

Table 2. Assessment of percentage differences in teeth reacting and not reacting to an air jet from a blower before (1) and directly after scaling (2), with division into teeth with recessions and without recessions

				Response to stimulus (2)		Total	
				No	No Yes		
		No	N	129	24	153	
	Response to	INO	%	84.3%	15.7%	70.5%	
Without	stimulus (1)	Vag	N	22	42	64	
recession		res	%	34.4%	65.6%	29.5%	
	Total	Total		151	66	217	
	Total		%	69.6%	30.4%	100.0%	
With recession	D	No	N	38	6	44	
	Response to		%	86.4%	13.6%	52.4%	
	sumulus (1)	Vag	N	21	19	40	
		res	%	52.5%	47.5%	47.6%	
	Total		N	59	25	84	
			%	70.2%	29.8%	100.0%	

 $\begin{array}{l} \mbox{Without recession: } p_{McNemar} = 0.883, RR = 4.184 \ (2.782 - 6.291) \\ \mbox{With recession: } p_{McNemar} = 0.006, RR = 3.483 \ (1.547 - 7.844) \\ \end{array}$

during the first measurement was RR=3.852 foa all teeth and RR=43.483 for recessed teeth, whereas during the third examination the value equaled R=3.144 for all teeth and 1.956 for recessed teeth, where the risks were statistically significant.

Discussion

The results obtained in the current study regarding the initial percentage of sensitive teeth in patients with periodontitis (34.6%) are in conformity with the results obtained by Tamaro et al. (5), who

				Response t	o stimulus	
				(3	8)	Total
				No	Yes	
		No	Ν	124	29	153
	Response to	INO	%	81.0%	19.0%	70.5%
Without	stimulus (1)	Vag	N	18	46	64
recession		res	%	28.1%	71.9%	29.5%
T-4-1		.1	N	142	75	217
	101	11	%	65.4%	34.6%	100.0%
With recession	D	No	N	26	18	44
	Response to		%	59.1%	40.9%	52.4%
	sumulus (1)	Vaa	N	8	32	40
		Yes	%	20.0%	80.0%	47.6%
	Total		N	34	50	84
			%	40.5%	59.5%	100.0%

Table 3. Assessment of percentage differences in teeth reacting and not reacting to an air jet from a blower before (1) and two weeks after scaling (3), with division into teeth with recessions and without

Without recession: $p_{McNemar} = 0.144$, RR = 3.792 (2.641 – 5.444) With recession: $p_{McNemar} = 0.076$, RR = 1.956 (1.327 – 2.881)

observed in their study that initially 30% of teeth qualified for non-surgical periodontal treatment react to an air-jet or tactile stimulus.

Literature provides little clinical data on the percentage of sensitive teeth in general population, because such studies are mostly based on questionnaire surveys and they report hypersensitivity prevalence only as a percentage of patients reporting such ailment. In the authors' own study (6), hypersensitivity was declared by 50% of patients visiting a periodontist, and by 75% of patients calling due to periodontal diseases. These results concur with research of other authors, who indicated that occurrence of hypersensitivity in patients visiting a periodontist is statistically significantly higher compared to patients attending a general dental clinic (7). The study by Fischer et al. (8) conducted in a clinic in Rio de Janeiro demonstrated that the occurrence of dentine hypersensitivity was related to earlier periodontal treatment.

It is interesting that the percentage of sensitive teeth decreased in the group of all teeth directly after scaling and polishing, however the difference was not statistically significant. In the group of teeth with recession, the percentage of sensitive teeth decreased significantly in the examination directly after scaling. The presence of recessions, which may result from too intensive tooth brushing, is often associated with an absence of dental deposits on a given tooth surface, therefore there is no need to use a scaling tip in this particular site. On the other hand, on surfaces with recessions, very thorough polishing is frequently performed. It must be stressed that in the current study scaling was conducted exactly according to certain rules, with the use of piezoelectric devices, with correct positioning of the working tip. Special attention was also paid to accurate polishing of tooth crowns and roots.

In the subsequent examination after two weeks, the percentage of sensitive teeth increased from 30.2 to 41.5% and the difference was statistically significant. The results are in concurrence with results reported by other authors. A meta-analysis of studies on occurrence of dentine hypersensitivity after periodontal treatment, which was performed by von Troil et al. (9), demonstrates that root dentine hypersensitivity increases from 9-23% before treatment to 54-55% after treatment. The highest increase of sensitivity occurs in examinations after 1-3 weeks, then it subsides in the next few weeks. An analysis of two examinations within the same study indicated that after root scaling and polishing, hypersensitivity of root dentine occured in about 50% of patients. In a study by Al-Sabbagh et al. (10), after a flap operation, root dentine hypersensitivity increased from around 30% to as much as 67-76% after one week. Then after six weeks the sensitivity fell to 45% even without therapy reducing dentine sensitivity. The increase of sensitivity one to three weeks after periodontal treatment can result from various causes.

General causes of increased dentine hypersensitivity in patients with periodontitis include exposure of root dentine - which is sensitive to external stimuli - resulting from alveolar bone loss in the course of periodontal disease. Moreover, periodontal procedures themselves, such as scaling or curettage, may lead to damage or loss of root cement or even of dentine surface layer, which in turn may cause increased permeability of exposed dentine not covered with cement, resulting in its hypersensitivity (11). The study by Rees et al. (12) demonstrated that ultrasonic devices remove 6.8 microns of tooth substance, whereas manual curettes remove 23.6 microns and sonic instruments from 12.5 to 18.8 microns. What is more, instrumentation with scalers may bring about coarseness in external layers of hard tissues of teeth. According to Jotikasthira et al. (13) sonic devices remove calculus the most accurately, but at the same time they cause the highest loss of tooth substance and surface coarseness.

Furthermore, a side effect of scaling, especially with regard to subgingival calculus and abundant dental deposits, may be not only loss of cement and dentine, but also loss of dentine smear layer. The presence of this layer is very important for the tooth as it closes dentine tubules and constitutes a barrier, thus decreasing dentine permeability and its hydraulic conductance. Accurate planing may cause abrasion of dentine's surface layer and initiate the creation of smear layer, which may lead to closure of open dentine tubules (14). As it was observed in the current study, thorough polishing may play an important role in hypersensitivity prevention directly after scaling.

Decreased hypersensitivity a few weeks after periodontal procedures may also result from accumulation of mineral substances from saliva (e.g. calcium carbonate) in the form of insoluble deposits in open dentine tubules, which leads to their gradual closure and reduction of fluid flow, and, consequently, to decreased sensitivity. These processes may be aided by preparations attenuating dentine sensitivity, applied by the dentist and the patient (15).

It should be pointed out that in the current study, due to performing of procedures refunded by the National Health Fund, no patients had their cleaned surfaces of teeth protected with fluoride varnish, as this procedure is not refunded, and only those patients were included in the study who did not consent to fluoridation. It might be expected that application of fluoride or other preparations decreasing dentine sensitivity might contribute to its reduction at a later stage.

Conclusions

Ultrasonic scaling may cause increased sensitivity of dentine. Thorough polishing may limit the occurrence of hypersensitivity directly after the scaling procedure. In order to limit post-treatment sensitivity, the operator should keep in mind the rules of correct work with ultrasonic scalers, he should remember about exchange of working tips, about adapting the power and cooling of the tip to individual conditions in patient's oral cavity – e.g. the amount and type of dental deposits or the presence of root surfaces not covered by bone or gingiva, as well as about performing appropriate polishing.

References

- 1. Zappa U, Smith B, Simona C, Graf H, Case D, Kim W. Root substance removal by scaling and root planing. J Periodontol. 1991 Dec;62(12):750-4.
- 2. Gantes BG, Nilvéus R, Lie T, Leknes KN. The effect of hygiene instruments on dentin surfaces: scanning electron microscopic observations. J Periodontol. 1992 Mar;63(3):151-7.
- 3. Brännström M, Johnson G, Nordenvall KJ :Transmission and control of dentinal pain: resin impregnation for the desensitization of dentin..J Am Dent Assoc. 1979 Oct;99(4):612-8.
- 4. Schiff T, Dotson M, Cohen S, De Vizio W, McCool J, Volpe A.: Efficacy of a dentifrice containing potassium nitrate, soluble pyrophosphate, PVM/MA copolymer, and sodium fluoride on dentinal hypersensitivity: a twelve-week clinical study. J Clin Dent. 1994;5 Spec No:87-92.

- Tammaro S, Wennström JL, Bergenholtz G., Rootdentin sensitivity following non-surgical periodontal treatment. J Clin Periodontol. 2000 Sep;27(9):690-7.
- Nędzi-Góra M, Górska R: Częstość występowania nadwrażliwości zębiny u pacjentów zgłaszających się do periodontologa. Magazyn Stomatologiczny 2012, 6, 70-73
- Taani SD, Awartani F.Clinical evaluation of cervical dentin sensitivity (CDS) in patients attending general dental clinics (GDC) and periodontal specialty clinics (PSC). J Clin Periodontol. 2002 Feb;29(2):118-22.
- 8. Fisher C. : Prevelence and distribution of cervical dentine hypersensivity in a population in Rio de Janerio., J.Dent, 1992, 20(5), 272-6
- 9. Von Troil B.: A systematic review of the prevelance of root sensivity fallowing periodontal therapy., J. Clin. Periodont., 2002, 29, supl.3, 173-177
- 10. All-Sabbagh M.: Incidnet and time course of dentine hypersensivity after periodontal treatment. J.Dent., 2010, 58,9-14
- Fogel H. M., Pashley D. H.: Effect of periodontal root planing on dentin permeability. J. Clin. Periodont., 1993, 20, 673-677.
- 12. Rees J. S., Addy M., Hughes J.: An in vitro assessment of the dentine lost during instrumentation using the Periosonic system. J. Clin. Periodont., 1999, 26, 2, 106-109.Jańczuk
- 13. Jotikasthira N. E., Lie T., Leknes K. N.: Comparative in vitro studies of sonic, ultrasonic and reciprocating scaling instruments. J. Clin. Periodont., 1992, 19, 560-569.
- 14. Z. Jańczuk Z.: Stomatologia Zachowawcza. PZWL, Warszawa 1999, 125-135/293-306
- 15. Kleinberg I: SensiStat. A new saliva-based composition for simple and effective treatment of dentinal sensitivity pain. Dent Today. 2002 Dec;21(12):42-7.

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The relationship between breastfeeding and the risk of maternal obesity

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Abstract

Objective: The aim of this study was to investigate the relationship between breatsfeeding and the risk of maternal obesity.

Methods: This study is designed as a societybased, descriptive and cross-sectional one and it aims at identfying the risk factors associated with maternal obesity and their relationship with breastfeeding.

Results: Of all the participants, 85.6 % of them are housewives and 46.9 % of them are literate or graduates of primary school. Besides, 48 % have low income and 81.4 % of live in a nuclear family. It was detected that 25.6 % of the women were obese according to their BMI before pregnancy. The average age of the women whose BMI is in the obese group (24.16 \pm 4.76) are higher than those who are not obese (23.05 \pm 3.51) (p<0.01). No relationship was detected between obesity and occupation, income, type of delivery, and pregnancy preference (p>0.05).

Conclusion: This study detected that one fourth of the women was obese. Obese women were found to use mixed feeding more often than the women who are not obese, to have lower education levels, and more children. Therefore, health care personnel should be more responsive to the potential breastfeeding problems of obese women and help controlling weight in women having multiple births.

Key words: breastfeeding, obesity, BMI.

Introduction

Today, obesity is one of the most important health problems of the developed and developing countries (1). In the last two decades, similar to modern west countries, Turkey also has revealed an increase in the ratio of obesity with the changes in socio economical levels and eating habits (2). Obesity is more common in women than men (1). It is estimated that nearly 65 % of the women and 39 % of the men in the general population have low or moderate degrees of obesity. According to the results of the Population and Health study, 58 % of the women have BMI (Body Mass Index) over 25,0 and 23 % of them have BMI over 30. Therefore, it is very likely for health care personnel to serve women who are overweight or obese (2, 3). Obesity may bring serious health problems to women and fetus (1, 2). Besides, women's having several births being obese indicates that pregnancy can be a reason for obesity (2).

Breastfeeding is one of the most prominent factors contributing to the healthy development of the child. Starting breastfeeding early has many benefits for the mother's and baby's health (3). Maternal obesity can have negative effects not only in the postpartum period but also in the lactation process. This case is thought to be resulted from the improving endocrine changes due to obesity (1, 4, 5). Hilson et al (2004) indicate that obese primipara women start to produce milk 72 hours later than those who are not obese (6). Liu et al (2010) detected that obese women gave up breastfeeding earlier than those who were not obese. Obesity can differ depending on the society and on the factors such as increasing age, low education level and increasing number of pregnancies (7).

Obese women need more health care services to maintain breastfeeding. More studies identifying how exactly breastfeeding affects obesity are required for the identification of these needs. There is lack of studies on the issue of the relationship between breastfeeding and obesity in our country. Therefore, this study aims to identify the risk factors associated with maternal obesity and their relationship with breastfeeding.

Material and Methods

This study is designed as a society-based, descriptive and cross-sectional one and it aims

at identfying the risk factors associated with maternal obesity and their relationship with breastfeeding. The study was conducted at a Primary Health care center in a city located in the eastern part of Turkey. All the individuals in Turkey are registered in the Primary Health Care Center records. People are provided protective health care services based on these records that are updated in every 6 months. The vaccination schedule of the babies is also arranged in these centers. The health care personnel visit mothers who do not bring their babies for vaccination in their houses. There are 24 Primary Health Care centers in the city center. These health care centers were grouped according to their size, transportation difficulty and socio economical level of the women and one health care center was chosen randomly. The data were collected in the vaccination polyclinic of this health care center between 2nd of February and 20th of July, 2010. Women having a 0-1 year old baby (n=443) were involved in the study. However, 52 of them having BMI below <18.5 were excluded. Hence, the study was carried out with 391 women.

Those not being with their babies due to some health problems after birth (premature baby, cleft palate or lip, etc), having a disease disabling breastfeeding (e.g. mammary abscess, etc), not knowing or remembering their weight before birth, and having multiple pregnancies were excluded from the study.

Instruments

The data were collected through the Participant Information form developed by the researcher in the light of the related literature (3, 6, 8-12). The data were collected from the women who accepted to participate in the study through the interviews by the researcher. Collection of the data was performed before the babies were vaccinated to create a more comfortable atmosphere in the data collection process. The Participant Information Form was piloted with 20 women and revised before it was used in the actual study. Those having health problems in their pregnancies (n:5), not having enough time to allocate for the study (n:15), and thinking that their husbands would not allow them to participate in the research (n:10) did not accept to be involved in the study.

The Participant Information Form was composed of questions related to the personal characteristics of the women (age, occupation, level of education, income, family type, weight), fertility characteristics (number of births, type of birth, pregnancy preference, weight gained in pregnancy), and breastfeeding practices (breastfeeding after birth, time to start breastfeeding, breastfeeding at the present, type of feeding, time to start supplemental feeding). State of breastfeeding encompasses the last 24 hours. In exclusive breastfeeding, the infant receives only breast milk and is allowed supplementation of drops and syrups such as vitamins, minerals, and medicine. In mixed feeding, which is also called supplemental feeding in Turkey, some food (e.g. water, water with sugar, honey, jam, tea, linden tea, daisy tea, fennel tea, cow's milk, and Formula) is given together with the breast milk (3). The participants' height was measured using a measuring tape in the health care center and their BMI was calculated using the Statistical Package for the Social Sciences 10.0 (SPSS 10.0). Women with a BMI \geq 30 were considered obese. According to the WHO criteria, women with < 18.5 BMI are considered thin, 18.5-24.99 normal, 25.0-29.00 stage I obese, 30.0-39.99 stage 2 obese, and \geq 40 stage III obese. Those with \geq 30 BMI are accepted as obese. One of the most important limitations of the study is the probability of women's not recalling their exact weight before pregnancy.

Ethical Considerations

The official permissions were obtained from the health care center where the study would be conducted. The women were informed by the researcher that they would not be paid for their participation in the study and they did not receive any payment.

Statistical analysis

The data were analyzed using the Statistical Package for the Social Sciences 10.0 (SPSS 10.0). Demographic data and independent variables were analyzed using descriptive statistic techniques. The data were analyzed using the student's t-test, ² and backward stepwise logistic regression, and p values <0.05 were considered significant. Backward Stepwise Logistic Regression analysis

Characteristics	Normal (275)	Obese (116)	Total (n:391)
+Age of the mothers			
$(Mean \pm SD)$	23.05 ± 3.51	24.16 ± 4.76	≠P:0.001
	n (%)	n (%)	n (%)
Occupation			
Housewife	231(84.0)	104(89.7)	NS
Working out of home	44(16.0)	12(10.3)	
Level of education			
Literate / Primary school	116(42.2)	68(58.6)	
High school or University	159(57.8)	48(41.4)	≠P:0.001
Family income			
Low	128(46.5)	60(51.7)	NS
Medium / Good	147(53.5)	56(48.3)	
Family type			
Nuclear family	227(82.5)	92(79.3)	
Large family	48(17.5)	24(20.7)	§P:0.002
Number of births			
1	127(46.2)	36(31.0)	
2≥	148(53.8)	80(69.0)	§P:0.006
Type of birth			
Vaginal	144(52.4)	56(48.3)	
Cesarean	131(47.6)	60(51.7)	NS
Pregnancy preference			
Wanted	239(86.9)	108(93.1)	
Unwanted	36(13.1)	8(6.9)	NS
Total	275 (74.4)	116 (25.6)	

*Table 1. Characteristics of the participants stratified by maternal pre-pregnant obesity** (N: 391)

+Average Age: 27.31 ± 4.96 (Min:18; Max: 43)

*Body Mass Index (BMI) is categorized according to the WHO. Continuous data are presented as mean \pm SD, and categorical data are presented as percentages (number of valid observations). Differences assessed by Student's t-test and $\chi 2$ test. § P < 0.01, $\neq P < .0001$

was conducted for the significant variables according to bivariate analysis results (13).

Results

The average age of the participants is 27.31 ± 4.96 . Of all the participants, 85.6 % of them are housewives and 46.9 % of them are literate or graduates of primary school. Besides, 48 % have low income and 81.4 % of live in a nuclear family. It was detected that 25.6 % of the women were obese according to their BMI before pregnancy. Table 1 displays the characteristics of the women according to their state of obesity before pregnancy. The average age of the women whose BMI is in the obese group (24.16 ± 4.76) are higher than those who are not obese (23.05 ± 3.51) (p<0.01). BMI decreases with the increase in the level of

education. While the percentage of obesity is 58.6 % in women who are literate or graduates of primary school, this proportion is 41.4 % among the graduates of university (p<0.05). The frequency of obesity is less in those living in a nuclear family than those of in large families (p<0.05). In addition, obesity was found to increase with the increase in the number of births (p<0.05). No relationship was detected between obesity and occupation, income, type of delivery, and pregnancy preference (p>0.05).

Table 2 demonstrates the relationship between the breastfeeding variables and obesity before pregnancy. Of all the participants, 95.9 % of them reported to have breastfed their babies after birth and 66.2 % of them breastfed in the first one hour after birth. Besides, 90.8 % of the women stated that they were still breastfeeding their babies. Howe-

Breastfeeding practices	Normal (275) N (%)	Obese (116) N (%)	Total N (%)	Р
Breastfeeding after birth				
Yes	263 (95.6)	112 (96.6)	375 (95.9)	
No	12 (4.4)	4 (3.4)	16 (4.1)	NS
⁺ Time to start breastfeeding				
Within the first hour of birth	179 (65.1)	80 (69.0)	259 (66.2)	
1 hour after birth	96 (34.9)	36 (31.0)	132 (33.8)	NS
State of breastfeeding				
Still going on	251 (91.3)	104 (89.7)	355 (90.8)	NS
Not anymore	24 (8.7)	12 (10.3)	36 (9.2)	
Breastfeeding practices				
Exclusive breastfeeding	132 (48.0)	40 (34.5)	172 (44.0)	
Mixed feeding	143 (52.0)	76 (65.5)	219 (66.0)	¥ P:0.05
	Mean \pm SD	Mean \pm SD	Mean \pm SD	
*Time to start supplemental feeding (months) (n:243)	4.25 ± 2.18	4.35 ± 2.45	4.15 ± 2.26	NS
≠Weight gained in pregnancy (n:355)	10.50 ± 14.26	9.41 ± 9.36	10.47 ± 14.24	NS

Table 2.Bivariate relationships between breastfeeding practices and maternal pre-pregnant obesity (N: 391)

* Only those who started supplemental feeding answered

 \neq Those knowing their weight before and after pregnancy answered.

Differences assessed by χ^2 test and Student's t-test, $\neq P < 0.05$

*Table 3. Risk factors for obesity according to logistic regression model** (*N*=391)

Factors	β	SE ^a	df ^b	р	OR ^c	95% Cl ^d
Breastfeeding practices (referent: Exclusive						
breastfeeding)						
Mixed feeding	.488	.236	1	.039	1.63	1.026-2.588
Educational level (referent: High school or						
university)						
Literate /Primary school	.500	.232	1	.032	1.64	1.045-2.599
Number of births (referent:1)						
2≥	. 572	.240	1	.017	1.77	1.107-2.837

* Backward Stepwise Logistic Regression

SEa: Standard Error; dfb: Degree of freedom; ORc: Odd's ratio; Cld: Confidence interval

ver, no relationship was detected between obesity and the time to start breastfeeding and to give supplemental feeding (p>0.05). In addition, no significant relationship was found between obesity and the weight gained in pregnancy (p>0.05).

Forty-four per cent of the participants provided exclusive breastfeeding to their babies. Obesity among those who provided exclusive breastfeeding was 34.5 % while it was 65.5 among those who used mixed feeding (p<0.05).

Table 3 displays the Backward Stepwise Logistic regression analysis results based on the model created depending on the relationship between obesity and age, education level, family type, number of births and type of feeding. It was found that obese women tend to use mixed feeding 1.63 times more frequently than those who are not obese, they are literate or graduates of primary school 1.64 times more commonly than others, and have multiple deliveries (2 and more) 1.77 times more commonly than those who are not obese.

Discussion

Today, obesity is one of the most prominent problems in the developed and developing countries. Obesity increases in women in their reproductive age, which can cause problems related to birth and postpartum period. This study detected that one in every four women was obese. In Tur-

countries (10, 12, 16). The prevalence identified in this study is lower when compared to the obesity prevalence in other countries. Although obesity differs depending on the society, it is affected by factors such as life style, nutritional habit, and perception of the body image (1). The difference between the findings might have resulted from these factors. The average age of the obese women in this study was higher than those who were not obese. However, age was not detected a risk factor in the logistic regression analysis. Turkey Population and Health Research 2008 (TDHS) data indicate that BMI rapidly increases with age and the obesity prevalence is more than 25.0 % in women 25 years old and over (3). Aktener et al. (2006) found that obesity prevalence were two times higher in women after menopause than those in their reproductive age; however, age was not detected a risk factor in logistic regression model (15). Kugyelka et al. (2004) also found that the average age of the obese women (26.4 ± 4.9) were higher than those who were not obese (25.0 \pm 5.4) (10). Besides, the literature encompasses many studies demonstrating that obesity increases with age (7, 12, 14, 16-18). Findings of this study correlate with the ones in the literature.

key, obesity prevalence ranged between 22.2 %

and 33.9 % in the studies conducted with women

in their reproductive age (7, 14, 151). Findings of this study correlate with those of in these studies.

Obesity prevalence was detected between 30.7 % and 55.3 % in the studies conducted in different

This study reveals that women who are literate or graduates of primary school tend to develop obesity 1.6 times more than the ones who graduated from high school or university. TDHS (2008) data showed that women with low education levels had higher BMI than women who graduated from high school or university (3). While the literature encompasses several studies demonstrating the negative relationship between education level and BMI (9, 11, 12, 17), there are some other studies revealing no relationship between these variables (10, 14, 15, 19). Koruk and Sahin (2005) found that women with low education level tend to develop obesity 2.7 times more frequently than women with higher education level (7). Knowledge and thinking competence gained through education have effects on preventing body weight in adulthood and handling overweight (7). Women living in a large family have more births and lower education level (3). Therefore, the prevalence of obesity in this study was detected lower in women living in a nuclear family (p<0.05). However, family type was not accepted as a risk factor in the logistic regression analysis. Erkol and Khorshid (2004) detected no significant relationship between obesity and family type. Findings of this study are correlated with the ones in the literature (19). Several studies reveal the effect of number of births on obesity (7, 10, 12, 14-16, 18). This study indicated that obesity prevalence increases with the increase in the number of births. In their study conducted in 2007, Hajian-Tilaki and Hiedari found that each birth increases the risk of obesity 7 times (17). Despite the different proportions of the effect of number of births on the development of obesity, findings of this study are correlated with the ones in the literature.

No relationship was detected between obesity and occupation/income. The literature encompasses studies revealing a relationship between occupation and obesity (14, 19) as well as the ones which found no relationship (15, 17). Similarly, some studies identified a relationship between income and obesity (14) while some others did not (19). Findings of this study are similar to the ones in the literature. Obese pregnant women more likely experience complications caused by fetal macrosomia; for example dysfunctional action, prolonged birth action, bladder-perineum traumas and shoulder dystocia (1). Therefore, caesarean section is more frequently preferred in obese pregnant women. The literature contains many studies revealing the relationship between obesity and caesarean section (9, 10, 12). Dempsy et al. (2005) found that obese women are two times more at risk of having caesarean section than women who are not obese (20). This study detected no relationship between type of birth and obesity. The difference might have resulted from the differences in reasons while deciding to have caesarean. Thus, one of the most important reasons of having caesarean is the women's own preference (21). Breastfeeding is quite common in Turkey; even there are some minor differences in the basic characteristics, 97% of the babies are breastfed at least for some time (3). Almost all of the women in this study stated

that they breastfed their babies after birth and they were still breastfeeding. Findings of this study are similar to the data in TDHS 2008.

TDHS (2008) data show that 39% of the women started breastfeeding in the first one hour after delivery (3). This percentage is almost two times higher in this study. The difference is considered to be resulted from the region. Hence, babies born in the eastern part of Turkey are breastfed 2-3 months longer than the babies in other parts of the country (3).

Obesity can negatively affect both starting to breastfeed and going on breastfeeding. This situation can result from the changes in endocrine. Adipose tissue acts as a reservoir for steroid hormones and is a site of steroid production and metabolism. Therefore, progesterone level increases in obese women, which prevents production of prolactin. It was found that obese women in this study used mixed feeding 1.6 times more than the women who are not obese. It is thought that this situation is caused by the need for supplemental feeding as a result of lack of milk due to obesity. Hatsu et al. (2008) found that BMI of the women using mixed food was higher than those who exclusively breastfed (8). Baker et al. (2004) detected a significant relationship between obesity and starting supplemental feeding (9). Kugyelka et al. (2004) found that obese Hispanic women have 1.5 times more difficulty breastfeeding exclusively (10). Findings of this study support the ones found in Hatsu et al. (2008), Baker et al. (2004) and Kugyelka et al. (2004).

The literature encompasses many studies detecting a negative relationship between obesity and breastfeeding practices (6, 9-12, 16, 22-24). In addition, Hilson et al (1997) detected no relationship between BMI and starting to breastfeed (25). This study found no relationship between obesity and breastfeeding after birth, time to start breastfeeding or supplemental food and weight gained in pregnancy. Findings of this study correlate with those of Hilson et al. (1997) but differ from the other studies (25). The difference might have resulted from the lower percentage of obesity compared to other countries and better breastfeeding variables in the country in general. Besides, there is a need for more studies identifying the relationship between BMI and obesity.

Conclusion

This study detected that one fourth of the women was obese. Obese women were found to use mixed feeding more often than the women who are not obese, to have lower education levels, and more children. Therefore, health care personnel should be more responsive to the potential breastfeeding problems of obese women and help controlling weight in women having multiple births. Despite the large number of obese women in our country, more studies are needed to identify the relationship between obesity and breastfeeding. This study is thought to shed light to the studies related to this issue but more studies are needed.

References

- 1. Apay SE, Kılıç M, Pasinlioğlu T. Obez labor and postpartum period in obese pregnant. TAF Prev Med Bull 2010; 9(2):151-6.
- 2. Apay SE, Pasinlioğlu T. Obesity and pregnancy. Prev Med Bull 2009; 8(4):345-50.
- 3. Turkey Demography and Health Survey. Hacettepe University. Prime Ministry Turkish Statistical Institute (TDHS), Ankara, 2008.
- Kulie T, Slattengren A, Redmer J, Counts H, Eglash A, Schrager S. Obesity and women's health: an evidence-based review. Am Board Fam Med 2011; 24:75–85.
- 5. Amir LH, Donath S. A systematic review of maternal obesity and breastfeeding intention, initiation and duration. BMC Pregnancy and Childbirth 2007;7: 9.
- 6. Hilson JA, Rasmussen KM, Kjolhede CL. High prepregnant body mass index is associated with poor lactation outcomes among white, rural women independent of psychosocial and demographic correlates. J Hum Lact 2004; 20(1):18-29.
- Koruk İ, Şahin TK. Prevalence and risk factors of obesity among 15-49 years-old house-wives in Fazilet Uluışık Health District in Konya. Genel Tıp Derg 2005; 15(4):147-155.
- 8. Hatsu IE, McDougald DM, Anderson AK. Effect of infant feeding on maternal body composition. Int Breastfeed J 2008; 6(3):18.

- 9. Baker JL, Michaelsen KF, Rasmussen KM, Sørensen TI. Maternal prepregnant body mass index, duration of breastfeeding, and timing of complementary food introduction are associated with infant weight gain. Am J Clin Nutr 2004; 80(6):1579-88.
- Kugyelka JG, Rasmussen KM, Frongillo EA. Maternal obesity is negatively associated with breastfeeding success among Hispanic but not Black women. J Nutr 2004; 134(7):1746-53.
- 11. Hilson JA, Rasmussen KM, Kjolhede CL. Excessive weight gain during pregnancy is associated with earlier termination of breast-feeding among White women. J Nutr 2006; 136(1):140-6.
- 12. Liu J, Smith MG, Dobre MA, Ferguson JE. Maternal obesity and breast-feeding practices among white and black women. Obesity (Silver Spring) 2010; 18(1):175-82.
- 13. Büyüköztürk, Ş. Handbook Data Analysis for The Social Sciences. Ankara: Pegem Yayıncılık. 2007.
- 14. Okyay P, Uçku R. Prevalence and risk factors of obesity of reproductive age women in an urban area of İzmir. ADÜ Tıp Fakültesi Dergisi 2002; 3(3):5-12.
- Aktener AY, Dülger Hİ, Erkayhan GE, Görmeli G, Kafadar S, Yıldız M. Obesity prevalence in reproductive age and postmenopausal women aged between 20-64 years in a semi-urban area. Trakya Univ Tip Fak Derg 2006; 23(3):119-26.
- 16. Kitsantas P, Pawloski LR. Maternal obesity, health status during pregnancy, and breastfeeding initiation and duration. J Matern Fetal Neonatal Med 2010; 23(2):135-41.
- 17. Hajian-Tilaki KO, Hiedari B Association between parity, live birth and the risk of obesity in women. Int J Endocrinol Metab 2007;4:109-18.
- 18. Sebire NJ, Jolly M, Harris JP, Wadsworth J, Joffe M, Beard RW, et al. Maternal obesity and pregnancy outcome: a study of pregnancies in London. International Journal of Obesity 2001; 25:1175–82.
- 19. Erkol A, Khorshid L. Obesity; predisposing factors and evaluation of the social dimension. SSK Tepecik Hast Derg 2004; 14(2):101-7.
- 20. Dempsey JC, Ashiny Z, Qiu CF, Miller RS, Sorensen TK, Williams MA. Maternal pre-pregnancy overweight status and obesity as risk factors for cesarean delivery. J Matern Fetal Neonatal Med 2005;17(3):179-85.

- 21. Şahin NH. Seksio-cesarean: The prevalence and consequences. Maltepe Üniversitesi Hemşirelik Bilim ve Sanatı Dergisi 2009; 2(3):93-8.
- 22. Theofilogiannakou M, Skouroliakou M, Gounaris A, Panagiotakos D, Markantonis SL. Breast-feeding in Athens, Greece: factors associated with its initiation and duration. Journal of Pediatric Gastroenterology and Nutrition 2006; 43:379-84.
- 23. Krause KM, Lovelady CA, Ostbye T. Predictors of breastfeeding in overweight and obese women: data from active mothers postpartum. Matern Child Health J 2011; 15(3):367-75.
- 24. Donath SM, Amir LH. Does maternal obesity adversely affect breastfeeding initiation and duration? J. Paediatr. Child Health 2000; 36:482–6.
- 25. Hilson JA, Rasmussen KM, Kjolhede CL. Maternal obesity and breast-feeding success in a rural population of white women. Am J Clin Nutr 1997; 66:1371-8.

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The value of serum brain natriuretic peptide for hyperbaric oxygen treatment in CO poisoning

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Abstract

Objective: The aim of this study was to investigate the serum BNP levels in patients with acute CO poisoning who received HBO treatment, and to compare them those in patients with acute CO poisoning who received NBO treatment.

Methods: The patients were divided into 2 groups. Group 1 consisted of patients treated with hyperbaric oxygen, whereas the patients in group 2 treated with normobaric oxygen. The serum BNP levels were compared in group1 and group 2. p-values lower than 0.05 was accepted as statistically significant.

Results: 94 patients were enrolled in the stud Median BNP level was 96.3 pg/ml in group 1 and 31.8 pg/ml in group 2. There was significant difference in groups (p<0.05). A cut-off of 43.1 pg/ml yielded a sensitivity of 87.5% and specificity of 69.4%.

Conclusion: We suggest BNP levels, should be used as a marker for HBO treatment in carbon monoxide-poisoned patients

Key words: Emergency, BNP, CO poisoning.

Introduction

Carbon monoxide (CO) is produced by incomplete combustion of carbon containing compounds and toxicity results in cellular hypoxia. At the early phase of the leakage, prevention from toxicity is difficult due to its colorless and odorless properties (1). CO poisoning is one of the most common types of intoxication, and it is the leading cause of death by poisoning in the world (2,3). Suicides and fires are the main causes of CO toxicity in developed countries but in our region leakage of the gas from stoves and heaters are the main causes CO poisoning is a hypoxic lesion caused by replacement of oxyhemoglobin (O2Hb) by carboxyhemoglobin (COHb). It binds to hemoglobin 230 to 270 times rapidly than oxygen resulting in hypoxemia and resulting a leftward shift at the oxyhemoglobin dissociation curve that decreases the availability of oxygen to the already hypoxic tissues (1,4). These characteristics of CO and COHb makes most vulnerable organs of the body such as heart and brain, while they have the highest oxygen requirement in the organism. Cardiotoxic effects of CO poisoning is not only due to hypoxemia but also CO binds to myoglobin in heart and muscles that may generate an additional effect on myocardial depression. CO poisoning may cause transient left ventricular ejection fraction depression, valvular dysfunction and arrythmia (5-7). Weaver at al show Hyperbaric Oxygen (HBO) treatment was a significant reduction in neurologic sequelae (8). Kao et al mentioned that emergency physicians could identified patients receiving HBO therapy (9).

B type natriuretic peptide (BNP) is a member of a family of 4 human natriuretic peptides that share a common 17-peptide ring structure and released from myocardial cells in response to increased volume expansion and wall stress (10). It was shown to be 10-fold more abundant in the heart than brain (11). The aim of this study is to investigate the serum BNP levels in patients with acute CO poisoning and to compare that who received NBO treatment or HBO treatment. Thus serum BNP levels could be used as a marker of HBO treatment.

Methods

Study population

After approval by the local ethical committee, patients admitted to the Emergency Department (ED) between December 2010 and January 2012 with the diagnosis of carbon monoxide poisoning were enrolled. This cross-sectional prospective study performed multi-centrically. Patients were enrolled if they had a documented exposure to CO, elevated COHb levels, and a characteristic clinical presentation. The inclusion and exclusion criteria are shown in Table 1. The control group consist of thirty healty volunteer adults.

Procedures

Physical examinations were performed at admission. Blood BNP level were analyzed immediately. The BNP concentrations were measured with a fluorescence radioimmunoassay device (Biosite Incorporated® California, USA). The detection lower limit of the assay is 5 pg/ml. We compared that control group and patient group for BNP levels. After this patient group were divided into 2 new groups. Group 1 consisted of patients treated with hyperbaric oxygen, whereas the patients in group 2 treated with normobaric oxygen. Indications for referral for HBO treatment are shown in Table-2 (10). The serum BNP levels were compared in group 1 and 2.

Statistical analyse

Statistical analyses were performed using SPSS 15.00 program. Demographic and clinical features of the patients were examined according to mean \pm SD, median, range, and percentage. The normal distributions were tested with Kolmogorov-Smirnov test. Mann-Whitney U-test was used used for 2 continuous group comparisons. Chi-square (X2) test was used for categorical variables. Receiveroperating characteristic (ROC) curve was used to determine the BNP. p-values lower than 0.05 was accepted as statistically significant.

Results

Thirty healty volunteer and 94 patients poisoned by CO (mean age 43.64±15.99, 44.7% male) were enrolled in the study. Median BNP level was 43.1 pg/ml in patients group and 5.0 pg/ml in control group. There was significant difference in groups (p < 0.05). There were no suicidal attempts. Headache (51%) and nausea (36%) were the most common symptoms in patients admitted with CO poisoning. Symptoms of patient shown on the table-3. According to the groups the demographic and clinic characteristics of patients were summerized in the table-4. Median BNP level was 96.3 pg/ml in group 1 and 31.8 pg/ml in group 2. There was significant difference between two groups (p<0.05). Receiver operating characteristics curve

adie1. Inclusion and exclusion criteria of study in patients with CO poisoning					
Inclusion	Exclusion				
>18 years old	<18 years old				
poisoning with CO	Presentation after 24 hours of poisoning				
Presentation at first 24 hours of poisoning	Patients with heart failure				

Table 1 Induction and evolution optimic of study in particular with CO poisoning

Table 2. Commonly utilized indications for referral for Hyperbaric Oxygen Treatment

Syncope	nau
Confusion/altered mental status	hea
seizure	Syn
coma	seiz
Focal neurologic deficit	con
Pregnancy with carboxyhemoglobin level>15%	Foc
Blood level>25%	Preg
Evidence of acute myocardial ischemia	my

Table 3. Distribution symptoms of patients

	Case number (n)	%
nausea	34	36.2
headache	48	51.1
Syncope	17	18.1
confusion	21	22.3
seizure	5	5.3
coma	8	8.5
Focal neurologic deficit	9	9.6
Pregnancy	4	4.3
Evidence of acute myocardial ischemia	4	4.3

	Group1 (n=32)	Group 2 (n=62)	р
Sex (M/F)	8/24	34/28	0.06
Age (year, mean±SD)	37.69±14.1	45.9±16.2	0.57
SBP (mmHg mean±SD)	121.2±17.5	121.0±30.9	0.72
DBP (mmHg mean±SD)	72.8±10.2	70.0±10.2	0.67
pulse (/min)	83.1±9.8	79.2±14.5	0.46
COHb (%)	33.4±14.7	18.0±4.8	0.000
Bnp (pg/ml) (median. IQR)	96.3 (144)	31.8 (47.5)	0.000

Table 4. Characteristic of patient according to groups

was constructed to determine the BNP cut-off with highest combined sensitivity and specificity for HBO treatment (Figure 1). Using a cut-off of 43.1 pg/ml, the sensitivity of BNP for HBO treatment was 87.5%, with a specificity of 69.4%. The area under the ROC curve was 0.75 (95% CI, 0.640-0.860). One patient died in HBO treatment group. He has been already coma at admission.. All other patients in group 1 and 2 discharged without any sequellae.

Discussion

CO poisoning, a common cause of toxicological morbidity, is a potentially lethal disorder with immediate and delayed sequelae. Depending on the severity of exposure, patients with acute CO poisoning have various clinical manifestations ranging from mild symptoms such as headaches, dizziness, and impairment of higher cerebral function to severe symptoms such as mental confusion, collapse, convulsions, and paralysis (11). Previous researches were done with S-100B, NT-proBNP, Troponin-I in CO poisoning as a marker of diagnose. (4, 9,13, 14) It is well known that BNP is secreted primarily by the ventricular myocardium in response to wall stress and CO toxicity results with transient depression of ejection fraction as well as valvular dysfunction. In one study conventional markers together with ECG reported to be inadequate for detection of cardiotoxicity and NT proBNP levels assessed as a diagnostic marker of cardiotoxicity in patients with CO poisoning. (13). Recent studies reported that myocardial injury is the most important factor that predicts short and long term outcome in moderate to severely poisoned patients (9,15).

These findings make early detecting of myocardial injury in CO poisoning have a great



Figure 1. BNP-HBO tretment ROC Curve

importance while poor outcome negatively relates with the time interval form exposure to HBO treatment. Davutoğlu et al showed the levels of NT-proBNP and carboxi hemoglobin were increased in patients with CO poisoning and suggested that measuring the plasma NT-proBNP levels may contribute to early diagnosis of cardiotoxicity in patients with carbon monoxide poisoning(13). In our study, patients groups BNP levels increased consistently with the literature. Giannakoulas et al reported that NT-proBNP levels were raised in patients with acute ischemic stroke and suggested that neurohumeral activation occurred as a response to cerebral ischemia or direct myocardial dysfunction (14). Montaner et al (16) has reported that release of BNP from ischemic brain tissue is increased and it may be interrelated with the severity of brain ischemia. Laskowitz et al (17) demonstrated that during initial 24 hours following the ischemic stroke serum BNP levels increase, however they stated that since BNP is not specific to brain tissue, its use in panel test would be more accurate. İt welll known that HBO treatment used in neurological damage caused cerebral ischemia. So BNP may be a marker of cerebral ischemia

and HBO treatment. To the best of our knowledge, our study is the first study that examine BNP, as a biochemical marker for HBO treatment in the COpoisoned patients. Our study included 94 patients.

The age and sex differences were not significant in two groups. Nausea and headache were not significantly, confusion was significantly higher in group 1 patients than group 2. Pach et al showed BNP levels increased in all toxicated patients compared to control group (18). In our study, BNP levels were found significantly high in Group1 than Group 2. Using a cut-off of 43.1 pg/ml, the sensitivity of BNP for HBO treatment was 87.5%, with a specificity of 69.4%. Either as a marker of cardiac toxicity or as a response to cerebral ischemia in the setting of CO poisoning; cardiac natriuretic peptides particularly BNP should be used to guide oxygen treatment.

Conclusion

Myocardial toxicity may be subtle and is already present when patients are unconscious due to CO exposure and; in addition to hypoxic state of brain; reduced brain perfusion due to cardiotoxicity, may play an important role in unconsciousness of patients.

Our results showed that there were significant differences in serum BNP levels of patients who received HBO treatment. We suggest BNP levels, should be used as a marker for HBO treatment in carbon monoxide-poisoned patients.

References

- 1. Halebian P, Robinson N, Barie P, Goodwin C, Shires GT. Whole body oxygen utilization during acute carbon monoxide poisoning and isocapneic nitrogen hypoxia. J Trauma. 1986; 26:110-7
- 2. Hampson NB. Trends in the incidence of carbon monoxide poisoning in the United States. Am J Emerg Med 2005;23: 838-841,
- Cetin M, Ornek E, Sani NM, Cetin ZG, Oksuz F, Gokcen E. A Case of Carbon Monoxide Poisoning Presenting with Supraventricular Tachycardia. Internal Medicine 2012;51: 2607-9
- 4. Yardan T, Meric M, Bozkurt A, Bilge S, Bas DB, Bedir A, et al. The role of heart-type fatty acid-binding protein in the evaluation of carbon monoxide poisoning in rats. Hum Exp Toxicol. 2011; 30: 124-8. Epub 2010 Apr 7.

- Kalay N, Ozdogru I, Cetinkaya Y, Eryol NK, Dogan A, Gul I, et al. Cardiovascular effects of carbon monoxide poisoning. Am J Cardiol. 2007; 99: 322-4. Epub 2006 29.
- 6. Gandini C, Castoldi AF, Candura SM, Priori S, Locatelli C, Butera R, et al. Cardiac damage in pediatric carbon monoxide poisoning. J Toxicol Clin Toxicol. 2001; 39 : 45-51.
- San Lorenzo IS, Chiesa M, Gamba P, Toniolo A. Cardiologic aspects of carbon monoxide poisoning. Cardiologia. 1989; 34 : 439-46
- 8. Weaver LK, Hopkins RO, Chan KJ, Churchill S, Elliott CG, Clemmer TP et al: Hyperbaric oxygen for acute carbon monoxide poisoning. N Engl J Med 2002;347: 1057-1067.
- 9. Kao HK, Lien TC, Kou YR, Wang JH. Assessment of myocardial injury in the emergency department independently predicts the short-term poor outcome in patients with severe carbon monoxide poisoning receiving mechanical ventilation and hyperbaric oxygen therapy. Pulm Pharmacol Ther. 2009; 22: 473-7. Epub 2009 Apr 22
- 10. Wilkins MR, Redondo J, Brown LA. The natriureticpeptide family. Lancet. 1997; 349: 1307-10
- Pandit K, Mukhopadhyay P, Ghosh S, Chowdhury S. Natriuretic peptides: Diagnostic and therapeutic use. Indian J Endocrinol Metab. 2011;15 Suppl 4: 345-53.
- Maloney G. Carbon Monoxide. In:Tintinalli J, Stapczynski JS, Ma OJ, Cline DM, Cydulka RK and Meckler GD (Eds). Tintinalli's Emergency Medicine. A Comprehensive Study Guide.7th Ed. Mc Graw Hill New York 2010. p.1410-1413.
- Davutoglu V, Gunay N, Kocoglu H, Gunay NE, Yildirim C, Cavdar M, Tarakcioglu M. Serum levels of NT-ProBNP as an early cardiac marker of carbon monoxide poisoning. Inhal Toxicol. 2006; 18:155-8
- 14. Giannakoulas G, Hatzitolios A, Karvounis H. N-terminal pro-brain natriuretic peptide levels are elevated in patients with acute ischemic stroke. Angiology. 2005; 56:7 23-30.
- 15. Henry CR, Satran D, Lindgren B, Adkinson C, Nicholson CI, Henry TD. Myocardial injury and long-term mortality following moderate to severe carbon monoxide poisoning. JAMA. 2006; 295: 398-402.

- 16. Montaner J, Perea-Gainza M, Delgado P, et al. Etiologic diagnosis of ischemic stroke subtypes with plasma biomarkers. Stroke 2008;39: 2280-2287.
- 17. Laskowitz DT, Kasner SE, Saver J, Remmel KS, Jauch EC. Clinical usefulness of a biomarker-based diagnostic test for acute stroke.Stroke 2009; 40: 7785.
- 18. Pach D, Gawlikowski T, Targosz D, Groszek B, Wilimowska J. B-type natriuretic peptide plasma concentration in acutely poisoned patients. Inhal Toxicol. 2006;18:155-8.

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Ubiquity of Parasporin producers in Bacillus thuringiensis natural population of Iran

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Abstract

Bacillus thuringiensis, the most successful and most widely used microbial insecticide, products crystal proteins. Parasporin, a new crystal protein derived from non-insecticidal and non-hemolytic Bacillus thuringiensis, recognizes and kills human cancer cells. In this study, we characterized parasporin activities associated with one novel geographical isolates of B.thuringiensis. Characterization was performed based on biochemical tests, protein crystal morphology and variation of cry protein toxin using SDS-PAGE. Most isolates produced heterogenic and bipyramidal crystals. Upon processing of parasporin with proteinase K, the active form of 32 kDa was produced. Parasporal inclusion proteins of E-1 isolate were toxic to acute lymphoblastic leukemia cells (ALL), but not to normal lymphocyte. Our results provide evidence that the parasporin producing organism is a common member in B.thuringiensis populations occurring in natural environments of Iran.

Key words: Bacillus thuringiensis, Parasporin, human cancer cells, Cry proteins.

Introduction

Bacillus thuringiensis is, a Gram-positive bacterium, produces crystalline inclusion proteins during sporulation. Parasporal inclusion-forming organisms occur in a variety of aerobic and anaerobic species belonging to several genera, e.g. Bacillus, Paenibacillus, Brevibacillus and Clostridium ⁵⁻¹⁰⁻¹⁸. The crystal proteins are consisting of high specific insecticidal activities, for several orders and other invertebrates such as nematodes, mites and protozoa. This unique property has created B.thuringiensis an ecologically sound microbial control agent for insect pests of agricultural and medical significance ¹⁶⁻¹⁹. Earlier studies have shown that non-insecticidal B.thuringiensis strains are more greatly distributed than insecticidal strain in natural environment. Recently, Mizuki and his workers have found isolates of B.thuringiensis with a novel property, non-hemolytic and non-insecticidal, but interestingly, the inclusion proteins from some strains have shown specific cytotoxicity to some cultured cancer cells. These proteins could be used for medical aim and called "parasporin" ⁷⁻⁸⁻¹¹.

Cancer is a term for diseases in which abnormal cells divide without control and can invade other tissues. The cytocidal activity preferential for cancer cells make parasporin proteins possible candidates for anticancer agents of medical use ⁴⁻¹⁷. In this study we describe the cytotoxic activities of parasporin against ALL (Acute Lymphoblastic Leukemia) and normal lymphocyte. Parasporin proteins could be used for treatment of ALL in the future.

Materials and methods

Bacterial strains and culture media

The B.thringiensis used in this study were isolated from the soil samples collected in different geographic in Iran. The standard strain of B.thringiensis NCIMB 9134 was obtained from NCIMB, London. These strains were grown at 28°C on Nutrient agar (pH 7.6) consisting of meat extract (10 g), polypeptone (10 g), NaCl (2 g), agar (20 g) and distilled water (1000 ml) ³⁻¹⁹.

Human cells and culture conditions

The human cell line, ALL (Acute Lymphoblastic Leukemia) was purchased from the Institute Pasteur Cell Bank (Tehran, Iran). ALL cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and penicillin (100 units/ml)/streptomycine (100 μ g/ml) at 37°C (Katayama, 2005). Normal human T cells were freshly prepared from buffy coats by the method previously describe and were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and kanamycin (30 μ g/ml) at 37°C 12.

Parasporal inclusion proteins preparation, proteolytic processing and toxin activation

Sporulation cultures were washed in 1 M NaCl + 0.01% triton X-100 by centrifugation. Then they were washed three times with distilled water. To extract proteins, the mixture of spores and parasporal inclusions were suspended in 50 mmol 1-1 Na2Co3 (pH 10) + 10 mmol 1-1 dithiothreitol + 1 mmol 1-1 EDTA for 60 min at 37°C. After solubilization of the inclusions, the supernatant was passed through a membrane filter for sterilization. For analysis of proteolytic processing, soluilized proteins were treated with proteinase K (60 µg/ml) for 90 min at 37°C. After incubation, phenylmethylsulphonyl fluoride was added to stop the proteolytic reaction ⁶⁻⁹.

Analysis of parasporal inclusion protein by SDS-PAGE

The δ – endotoxin analysis was carried out by doing SDS-PAGE for characterization of B. thuringiensis using crystal suspensions. SDS-PAGE was performed as described by Laemmli (1970) using 4% stacking and 12% resolving gels. After electrophoresis, the gels were stained with 0.1% Coomassie blue R-250. The size of proteins was determined using molecular mass standards ¹².

Assay of cytocidal activity

The levels of cytotoxicity of activated proteins were measured with the MTT [3-(4, 5-dimethl-2thiazolyl)-2,5diphenyl-2H tetrazolium bromide] assay, a cell proliferation assay. A cell suspension (90 µl) containing 2×104 cells was delivered to each well of a microtest plate. After preincubation for 16 h at 37°C in a CO₂ incubator, then the well 10 µl of the inclusion proteins which had been prepared in 10-fold serial dilution at 37°C for 48 h. After incubation, the cytopathic effect (CPE) was monitored under a phase-contrast microscope, and to assess the level of cytotoxicity, MTT assay was done after incubation of the activated inclusion proteins. The absorbance of the converted dye was measured at 600 nm and the survival rate of the cells was calculated. The average absorbance of a mock-inoculated sample (100% cell survival) was taken as a negative control ³⁻⁷.

Results

Isolation of bacterial strains

Twenty soil samples were isolated for B. thuringiensis from 3 provinces of Iran. In total, 12 crystal forming B.thuringiensis strains isolated. Most of the B.thuringiensis isolates produced bipyramidal crystal and some of them roughly round and irregular-shape parasporal inclusion during sporulation when observed under light microscope (Fig. 1).

Analysis of parasporal inclusion protein by SDS-PAGE

Solubilization and proteolytic processing of the inclusion proteins have been shown in Fig 2. Parasporal inclusions of the standard strain B.thringiensis NCIMB 9134 and one of the isolated strains from soil contained different profile of proteins. B.thuringiensis isolates were characterized by SDS-PAGE of their crystal protein products and we selected 2 samples in this paper (Fig. 2). The results revealed that the isolated strain synthesize a group of proteins contained four major bands with molecular mass between 88, 81,



Figure 1. Photomicrograph of sporulating of *B.thuringiensis isolate*

37 and 20 kDa and several others proteins. The isolates produced 1-5 different protein bands and most of the isolates showed more than one band. In all of the isolates, after proteolytic digestion, the major band was 32 kDa (Fig.2).

Assay of cytocidal activity

Of the 12 B.thuringiensis strains with no hemolytic activity, one strain showed cytocidal activity against leukemia cells. The purified parasporal inclusion of one selected Iranian isolate named E-1 and was examined for its in vitro cytotoxic activity against ALL. After treatment of the parasporal inclusions of B.thuringiensis strains with proteinase K, measuring their cytotoxic effects on ALL and normal lymphocyte cells by the MTT assay. Fig 3 shows the cytotoxicity induced by proteinase K activated inclusion proteins from E-1strain.

Discussion

Bacillus thuringiensis is a major microorganism, which shows entamopathogenic, cytocidal and insecticidal activity. The organism is a ubiquitous, gram-positive and spore-forming bacterium that forms parasporal crystals during the stationary phase of its growth cycle ¹⁻²⁻¹⁵. In present study, 20 soil samples were collected from different region in Iran. The discrimination of Bacillus isolates were done according to their colony and cellular morphology. Based on the presence of crystalline inclusions is primary identification of B.thuringiensis 13. The morphology, size, and the number of parasporal inclusions may vary among the B. thuringiensis strains. However, the typical bipyramidal crystal and irregular were most in our results. Salehi and his workers reported bipyramidal crystals have shown most frequency in Iran¹⁴.

SDS-PAGE analysis is generally used to determine and compare protein profiles of B. thuringiensis isolates. B. thuringiensis can produce one or more crystalline inclusion; therefore, the protein profile of the organism shows differences among the strains. Another possible reason for the differences between protein profiles of the same Cry protein in two different studies could be that some environmental factors can turn on or turn off expression of some cry genes ¹⁶⁻²⁰.



Figure 2. SDS-PAGE of the B.thuringiensis strains parasporal proteins. Lane M, molecular standards. Lane1 and 3, alkali-solubilized inclusion proteins of B.thuringiensis strain. Lane 2 and 4 alkali- solubilized and protease K-treated inclusion protein of B.thuringiensis strains.



Figure 3. Cytopathic effect of proteinase-K digested proteins of Bacillus thuringiensis isolate E-1 on ALL cells 24 h (right) and 1 h (left) after treatment. ALL cells were observed with a phase-contrast microscope.

The 37 kDa protein is a protoxin, which is converted to the 32 kDa upon proteolytic processing. We investigated the proteolytic activation and cytocidal effects of parasporin, a novel B.thuringiensis parasporin toxin that was previously reported to kill human cancer cells. In this study indicates that the 32 kDa leukemic cellkilling protein, occurring in parasporal inclusions of B.thuringiensis strain E-1, unrelated to the hemolytic parasporal proteins of this bacterium. Ohaba and his workers reported, parasporin-2 is a polypeptide with a predicted molecular weight of 37,446. Proteolytic processing of the 37 kDa precursor protein yields a 30 kDa cytotoxin active on cancer cells10. It seems isolated protein in this research is parasporin-2. Alteration of the cytoskeleton structure was observed in the cells after exposure to the toxin. Parasporin-2 was found to be localized in the plasma membrane during the morphological changes that were correlated with increases in the plasma membrane permeability 6.

Jung and his workers isolated Cry31Aa2 and they reported it was cytocidal to some human cancer cells from different tissues but not to normal cells from same tissues4. In our study proparasporin is cleaved by proteinase K and converted into the potent toxin. This is toxicity on ALL cells and cause alteration in the cell morphology but absence of toxicity on normal T cells. Our results also suggest that the parasporal inclusion proteins of B.thuringiensis may provide a novel tool for expanded new diagnostic or anti-cancer agents.

Conclusion

Parasporal inclusion proteins produced by B. thuringiensis E-1 strain exhibit cytotoxicity against acute lymphoblastic leukemia cells when activated by protease treatment. The improvement of preparation method of the Parasporin might be useful for the other studies on the B.thuringiensis or recombinant proteins.

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References

- 1. Bel Y, Granedo F, Alverola TM, Martinez-Sebastian MJ, and Ferre J. Distribution, frequency and diversity of Bacillus thuringiensis in olive tree environment in Spain. Systematic of Applied Microbiology. 1997: 20; 652-658.
- 2. Chatterjee SN, Bhattacharya T, Dangar TK, and Chandra G. Ecology and diversity of Bacillus thuringiensis in soil environment. African Journal of Biotechnology. 2007: 13; 1587-1591.
- 3. Hayakawa T, Kanagawa R, Kotani Y, Kimura M, Yamagiwa M, Yamane Y, Takebe S, and Sakai H. Parasporin-2Ab, a newly isolated cytotoxic crystal protein from Bacillus thuringiensis. Current Microbiology. 2007: 55; 278-283.
- 4. Jung YC, Miauki E, Akao T and Cote JC: Isolation and characterization of a novel Bacillus thuringiensis strain expressing a novel crystal protein with cytocidal activity against human cancer cells. J. Appl Microbiol. 2007: 103; 65-70.
- 5. Kim HS, Yamashita S, Akao T, Saitoh H, Higuchi K, Park YS, Mizuki E and Ohba M. In vitro cytotoxicity of non-Cyt inclusion proteins of a Bacillus thuringiensis isolate against human cells, including cancer cells. J. Appl Microbiol. 2000: 89; 16-23.
- 6. Kitada S, Abe Y, Ito A, Shimada H, Kusaka Y, Matsuo Y, Katayama H, Okumura S, Akao T, Mizuki E, Kuge O, Sasaguri Y, Ohba M. Cytocidal action of parasporin-2, an anti tumor crystal toxin from Bacillus thuringiensis. J of Biological Chemistry. 2006: 281; 26350-26360.
- 7. Mizuki E, Park YS, Saitoh H, Yamashita S. Parasporin, a human leukemic cell-recognizing parasporal protein of Bacillus thuringiensis. Clinical and Diagnostic laboratory Immunology. 2000: 7; 625-634.
- 8. Mizuki E, Ohba M, Akao T, Yamashita S, Saitoh H, Park YS. Unique activity associated with non-insecticidal Bacillus thuringiensis parasporal inclusions: in vitro cell-killing action on human cancer cells. J. Appl Microbiol. 1999: 86; 477-86.
- 9. Namba A, Yamagiwa M, Amano H, Akao T, Mizuki E, Ohba M, Sakai H. The cytotoxicity of Bacillus thuringiensis subsp. coreanesis A1519 strain against the human leukemic T cell. Biochemica et Biophysica Acta. 2003: 1622; 29-35.
- 10. Ohba M, Mizuki E, Uemori A. Parasporin, a new anticancer protein group from Bacillus thuringiensis. Anticancer Res. 2009: 29; 427-33.

- 11. Okumura S, Akao T, Higuchi K, Saitoh H, Mizuki E, Ohba M, Inouye K. Bacillus thuringiensis serovar shadongiensis strain 89-T34-22 produces multiple cytotoxic proteins with similar molecular masses against human cancer cells. Letters in Applied Microbiology. 2004: 39; 89-92.
- 12. Poornima K, Selvanayagam P, Shenbagarathai R. Identification of native Bacillus thuringiensis strain from South India having specific cytocidal activity against cancer cells. J Appl Microbiol. 2010: 109;348-54.
- 13. Rampersad J, and Ammons D. Bacillus thuringiensis isolation method utilizing a novel stain, low selection and high throughput produced atypical results. BMC Microbiol. 2005: 24; 5:52.
- 14. Salehi Jouzani G, Pourjan Abad A, Seifinejad A, Marzban R, Kariman K, Maleki B. Distribution and diversity of Dipteran-specific cry and cyt genes in native Bacillus thuringiensis strains obtained from different ecosystems of Iran. J Ind Microbiol Biotechnol. 2008: 35; 83-94.
- 15. Santana MA, Moccia CC, Gillis AE. Bacillus thuringiensis improved isolation methodology from soil samples. Journal of Microbiological Methods. 2008: 75; 357-358.
- 16. Sevim A, Eryüzlü E, Demirba Z, Demir S. A Novel cry2Ab Gene from the Indigenous Isolate Bacillus thuringiensis subsp. kurstaki. J. Microbiol. Biotechnol. 2012: 22; 133–140.
- 17. Sheikh N, Masood M, and Naz N. Hematological and serological changes in the pre- and post-treatment breast cancer patients. 2011: Vol 5, NO 6, pp.1449-1457.
- 18. Travers RS, Martin PAW, Reichelderfer CF. Selective process for efficient isolation of Bacillus spp. Appl. Environ. Microbiol. 1987: 53; 1263-1266.
- 19. Uemori A, Maeda, Yasutake K, Ohgushi A, Kagoshima K, Mizuki E, Ohba M. Ubiquity of parasporin-1 producers in Bacillus thuringiensis natural populations of Japan. Naturwissenschaften. 2007: 94; 34-38.
- 20. Yamashita S, Katayama H, Saitoh H, Akao T, Park YS, Mizuki E, Ohba M, and Ito A. Typical three-domain cry proteins of Bacillus thuringiensis strain A1462 exhibit cytocidal activity on limited human cancer cells. J. Biochem. 2005: 138(6):663-72.

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Idiopathic menarche presenting at nine months of age: A case report

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Abstarct

Herein, we report a 9 month-old-girl who presented with breast enlargement and menstruation at 7 months old. All secondary causes of precocious puberty were ruled out by appropriate investigations. Patient was diagnosed as idiopathic precocious puberty. To the best of our knowledge, this is the earliest onset of primary isosexual true precocious puberty in literature. Idiopathic precocious puberty diagnosed in the first your of their life might be named differently.

Key words: Precocious, pubertal development, infantile.

Introduction

Genetic, nutritional, environmental, and socioeconomic factors affect complex biologic route of sexual development (Terasawa and Fernandez 2001; R.Rosenfield 2002; Delemarre-van de Waal 2005; Biro 2007). Although the mechanism of puberty is not known, a couple of factors including genetic, neurotransmitters and hormones are blamed for this issue. Initiation of puberty is the result of activation of pulsatile hypothalamic GnRH secretion (Terasawa and Fernandez 2001). Hypothalamic-pituitary-gonadal (HPG) axis during puberty is formed by hypothalamic GnRH release and, followed by pituitary gonadotropin secretion and gonadal steroid production (Ojeda, Lomniczi et al. 2006). The development of secondary sexual characteristics before the age of 8 in girls and 9 in boys is called precocious puberty (Papathanasiou and Hadjiathanasiou 2006). However, definition of precocious puberty depends on reliable population standards since ethnic and environmental factors are effective. There are two types of precocious puberty; one is central (gonadotropindependent) precocious puberty that results from premature activation of the hypothalamic-pituitarygonadal (HPG) axis and gonadotropin-independent precocious puberty. Central precocious puberty is idiopathic and generally seen among girls (Delemarre-van de Waal 2005; Papathanasiou and Hadjiathanasiou 2006). However, genetic factors play a key role in the timing of pubertal onset, where members of an ethnic group and mother-daughter, monozygotic-twin, and sibling pairs begin menarche at similar ages (Palmert and Boepple 2001). In this paper, we report a girl who developed all signs of isosexual puberty including breast enlargement, bone and somatic maturation, and menstruation at an extraordinarily early age of 9 months.

Case Report

A 9-month-old girl was presented to the obstetrics and gynecology department with the complaint of vaginal bleeding. On history, she had been breast budding for the last three months and also bled one more time when she was 7 months old. Each cycle lasted with modarete blood loss and a duration of 3-4 days. The patient was born 3000 grams with vaginal delivery at term after an uneventful pregnancy. Perinatal hypoxia or anoxia was not reported. The patient's mother has not used medicine during pregnancy and her menarche was at the age of 13 years. The history revealed that all physical and mental milestones had occured at the appropriate age. There was no history of any neurological deficit or febrile illness. On physical examination, the patient was 71.5 cm (>97th %), weight 9 kg (50-75th %) and head circumference was 45 cm (50-75th %). Her breast tissue was at stage III (Figure 1) and pubic hair was at stage I according to Tanner Classification. Genital examination was normal with her age and detailed neurological, abdominal and pelvic examinations were also normal.

Complete blood count, sedimentation rate, electrolytes and thyroid functions were normal. The left wrist X –rays for bone age detection was consisted with 2 year-old (Figure 2). Tumoral causes were tested and chorioembryonic antigen, alpha-fetoprotein and beta HCG levels were detected in normal range. Baseline LH, FSH and estradiol were measured by ICMA and considered to be pubertal. (FSH: 5.68 mIU/ml, LH: 2.27 mIU/ ml and estradiol: 90 pg/ml). GnRH test revealed LH peak level as 24 mIU/ml; LH/FSH peaks ratio after GnRH test was 2, and plasma estradiol consentrations was 90 pg/ml (55 pmol/L). Normal adrenal glands with no pelvic mass were detected with abdominal ultrasonography. Internal genitalias revealed right ovary as 26x 21x17 mm, left ovary as 22x16x18 mm diameter and uterus lenght as 47 mm which was considered as pubertal. Magnetic resonance imaging of the central nervous system (CNS) was normal other than hyperplasia of hypophysis that may the reason of puberty. Parenchymal length of hypophysis was 7 mm and there was not adenomateous apperance in hypophysis.

Idiopathic central precocious puberty was diagnoed with physical exam consistent with stage III telarche, high LH/FSH, estradiol levels and radiologic examinations that excluded tumoral and structural abnormalities. Therefore, a GnRH analogue (3.75 mg per month of a depot suspension of leuprolide acetate) was initiated and treated for 2 years. Growth monitoring before and 2 year after treatment is presented in growth chart (Figure 3). The diagnosis of central precocious puberty was established by an adequate response to the treatment. Breast development was regressed, growth velocity was decresed and bone maturation was ceased [six month progression in a year (fig 3)], size of the ovary was reduced and gonadotropin levels were returned to prepubertal along with estradiol level.

Discussion

Puberty that occurs early before 8 year of age for girls and 9 year for boys are called precocious puberty and seperated into gonadotropin-dependent disorders (true precocious puberty) and gonadotropin-independent disorders (precocious



Figure.1. Breast tissue consisted with Tanner III staging



Figure 2. Bone age was compatiple with 2 yearold when she was 9 months of age



Figure 3. Bone age was 2.5 years old, six-monthprogression in a year

pseudopuberty). The diagnosis of precoccious puberty is established with history, physical examination and laboratory after excluding exogeneous hormone exposure, CNS or systemic disease, pubertal history of family. Basal estradiol, testosterone, FSH, LH and progesterone concentrations followed by GnRH stimulation test are gold standard for the diagnosis. Pelvic, hypophysial MRI and abdomen ultrasonography can be helpful in etiology (R.Rosenfield 2002; Biro 2007). We diagnosed precocious puberty with history, physical exam, laboratory and screening tests. This case was a gonadothropin related central precocious puberty which was accompanied by 2 cyclic vaginal bleeding after 7 months of age. A structural central nervous system (CNS) abnormality can, however, be demonstrated in 25-75% of boys and in 8–10% of girls with central precocious puberty. Hypothalamic hamartoma, glioma, astrocytoma, and germinoma can cause precocious puberty; it can also occur in children with internal hydrocephalus or other lesions of the central nervous system, such as an earlier episode of meningitis or traumatic brain injury or prior radiotherapy to the head. Magnetic resonance imaging of the brain should be performed in order to search for a possible organic cause especially in boys, girls younger than 6 years old, girls with high estradiol levels (>30 pmol/L) and girls with rapid breast development (Carel and Leger 2008; Prete, Couto-Silva et al. 2008). We could not find a reson that explains the central early puberty.

Hypothalamic Gonadotropin Releasing Hormone (GnRH) secretory system activation starts onset of puberty. Mini puberty, temporarily activation of GnRH secretory network system, is seen during fetal-neonatal life and in the first 6 months of life. The significance of GnRH activation in these early periods is not known (Morris, Jordan et al. 2004). Activation of GnRH pulse generator activates pulsatile GnRH and subsequently episodic pituitary gonadotropin secretion that promotes normal gonadol development and function at puberty (Krsmanovic, Hu et al. 2009). Various neuropeptides and neurotransmitters like gluatamate, noradrenaline, g-aminobutyric acid-GABA, endogenous opiates, NPY, have roles in the regulation of GnRH neurons (El Majdoubi, Sahu et al. 2000). Kisspeptin, GPR54 and neurokinin B are reported to be the essential substances for the onset of puberty (Quaynor, Hu et al. 2007; Teles, Bianco et al. 2008; Clarkson, Boon et al. 2009).

A serine/threonine kinase mammalian target of rapamycin (mTOR) stimulates or blocks LH secretion by modulation of hypothalamic KiSS-1 (Roa, Garcia-Galiano et al. 2009). Though, neuroendocrine pathways that stimulates and suppreses puberty is complex. The factors affecting these pathways and mechanisms that regulate them are not yet fully understood.

Prepubertal vaginal bleeding is called menarche after excluding infection, foreign body, tumors and trauma (Pelletier, Foidart et al. 2009). Prepubertal vaginal bleeding should be evaluated for precocious puberty regardless of age, and other signs of puberty should also be sought. In this case, we diagnosed precocious puberty after excluding other causes of vaginal bleding. The case was 9 month old girl with vaginal bleeding. Central precocious puberty was diagnosed after laboratory tests results that were confirmed by response to LHRH suppresion test. The patient presented with the signs of progressive puberty immediately after mini puberty that suggested an abnormality in suppresion of mini-puberty. However, due to many pathophysiological factors, any work could not be done to support this hypothesis. Any factor affecting early activation of GnRH pulse generator may be another possible cause even if mini-puberty has been supressed. Two similar cases have been reported in the literature. Nienaber et al reported a 2 year old girl with pubertal findings and regular vaginal bleeding since 6 month old age. They diagnosed her as idiopathic central precocious puberty (Nienaber and van der Walt 1991). Rao et al has reported the second case which presented at 3 years old with secondary sexual characteristics and cyclic regular vaginal bleeding since 3 month of age. They reported her as an idiopathic. (Rao, Udwadia et al. 1987). In this case, the patient has been consulted by a physician in earlier age, started treatment earlier and has been followed for subsequent two years. Early treatment leads to more favorable psycosocial and physical response in those kinds of cases.

In conclusion, the importance of this case is that the patient has been presented with puberty at an early age immidiately after cessation of mini puberty. Although the exact role and physiopathology of mini puberty is not clear, our case may be beneficial in explaining the physiopathology. A more comprehensive studies and detailed genetic evaluation are needed for confirmation of these hypotheses concerning this period.

References

- Biro, F. M. (2007). "Puberty." Adolesc Med State Art Rev 18(3): 425-433, v.
- Carel, J. C. and J. Leger (2008). "Clinical practice. Precocious puberty." N Engl J Med 358(22): 2366-2377.
- Clarkson, J., W. C. Boon, et al. (2009). "Postnatal development of an estradiol-kisspeptin positive feedback mechanism implicated in puberty onset." Endocrinology 150(7): 3214-3220.
- 4. Delemarre-van de Waal, H. A. (2005). "Secular trend of timing of puberty." Endocr Dev 8: 1-14.
- El Majdoubi, M., A. Sahu, et al. (2000). "Neuropeptide Y: A hypothalamic brake restraining the onset of puberty in primates." Proc Natl Acad Sci U S A 97(11): 6179-6184.
- Krsmanovic, L. Z., L. Hu, et al. (2009). "The hypothalamic GnRH pulse generator: multiple regulatory mechanisms." Trends Endocrinol Metab 20(8): 402-408.
- Morris, J. A., C. L. Jordan, et al. (2004). "Sexual differentiation of the vertebrate nervous system." Nat Neurosci 7(10): 1034-1039.
- 8. Nienaber, C. S. and D. van der Walt (1991). "True idiopathic precocious puberty presenting at six months of age; a case report." Eur J Obstet Gynecol Reprod Biol 38(3): 243-245.
- 9. Ojeda, S. R., A. Lomniczi, et al. (2006). "Minireview: the neuroendocrine regulation of puberty: is the time ripe for a systems biology approach?" Endocrinology 147(3): 1166-1174.
- Palmert, M. R. and P. A. Boepple (2001). "Variation in the timing of puberty: clinical spectrum and genetic investigation." J Clin Endocrinol Metab 86(6): 2364-2368.
- Papathanasiou, A. and C. Hadjiathanasiou (2006). "Precocious puberty." Pediatr Endocrinol Rev 3 Suppl 1: 182-187.
- 12. Pelletier, R., J. M. Foidart, et al. (2009). "[How I explore ... the bleeding of vaginal origin in childhood]." Rev Med Liege 64(4): 219-222.

- 13. Prete, G., A. C. Couto-Silva, et al. (2008). "Idiopathic central precocious puberty in girls: presentation factors." BMC Pediatr 8: 27.
- 14. Quaynor, S., L. Hu, et al. (2007). "Expression of a functional g protein-coupled receptor 54-kisspeptin autoregulatory system in hypothalamic gonadotropin-releasing hormone neurons." Mol Endocrinol 21(12): 3062-3070.
- 15. R.Rosenfield (2002). Puberty in female and its disorders. Pediatric Endocrinology. M. A. Sperling. Philedelphia, Sounders Comp: 455-518.
- 16. Rao, P. P., Z. F. Udwadia, et al. (1987). "Precocious puberty presenting at 3 months of age." J Assoc Physicians India 35(11): 801-802.
- Roa, J., D. Garcia-Galiano, et al. (2009). "The mammalian target of rapamycin as novel central regulator of puberty onset via modulation of hypothalamic Kiss1 system." Endocrinology 150(11): 5016-5026.
- 18. Teles, M. G., S. D. Bianco, et al. (2008). "A GPR54activating mutation in a patient with central precocious puberty." N Engl J Med 358(7): 709-715.
- 19. Terasawa, E. and D. L. Fernandez (2001). "Neurobiological mechanisms of the onset of puberty in primates." Endocr Rev 22(1): 111-151.

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Neonatal hypernatremik dehydration awareness among primary care health givers

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Abstract

Introduction: Inadequate breastfeeding may cause critical infant morbidities such as neonatal hypernatremic dehydration (NHD). We aimed to assess the knowledge and attitude of family physicians and primary care nurses about the breastfeeding and NHD in primary care setting.

Methods: This is a cross sectional study carried out among 97 primary care nurses and 114 physicians practicing at the center of Malatya city were participated in this study.

Results: A total of 211 primary healthcare providers included in this study. Primary care physicians and nurses have not seen a case of NHD during their practice at the rate of 87.7% and 96.9% respectively. 55.7% of the nurses and 59.6% of the physician were of the opinion that 10% or more loss of the weight from birth weight indicates the inadequacy of breast milk. 61.4% of the physicians and 84.5% of the nurses either have no opinion or disagree to initiation infant formula when breast milk insufficient. The confidence of nurses and physician in the management of NHD in the primary care were 12.4% and 18.4% respectively.

Conclusion: This study remarks incomplete knowledge, awareness and attitude among primary care health care providers (nurse and physician) about NHD. We believe the emphasis on continuing medical education for health care providers would contribute to decrease NHD related morbidity and mortalities.

Key words: Primary care, hypernatremic dehydration, breastfeeding, newborn.

Introduction

The immediate and long term benefits of breastfeeding is well known and promotion of breast feeding has been the subject of concern for the pediatricians and family physicians (1, 2). The impact of health organizations efforts yielded increased rates of exclusive breast-feeding in developed as well as underdeveloped countries(3). The prevalence of infant morbidity caused by inadequate breastfeeding appears to be rising. Inadequate breastfeeding may cause critical infant morbidities such as neonatal hypernatremic dehydration (NHD) (4). Contrary to the past, hypernatremia occurring due to artificial feeds with high sodium concentration. Today insufficient lactation seems to be main cause of NHD (5). It was thought be an unusual cause of hospitalization of newborns not more than two decades ago. The incidence of this clinical entity was initially sporadic in early 1980s, nevertheless reports and trials showed that its prevalence is many fold greater then what is actually thought (6). Case reports and tertiary care studies point out an increase in the incidence and clinical significance of NHD associated with breastfeeding (7). Nevertheless it is encountered as a result of deprivation of water in infants if milk supply is insufficient in the first week of life (8).

Since causes of NHD are preventable and commonly first seen in the primary care, health care givers should pay more attention to the techniques of breastfeeding and immediate intervention in case of insufficiency of breast milk. Yet there are limited field based studies elaborating the primary care health providers approach to NHD. We aimed to assess the knowledge and attitude of family physicians and primary care nurses about the breastfeeding and NHD in primary care setting.

Methods

Study design ad Participants

This is a cross sectional study carried out among the family physicians and primary care nurses in Turkey. Family physicians serve an average of 3500-4000 population currently. They work in a family health care center which is easily accessible locations in city centers. Each family health care center includes 4-6 physicians, allied staff, and 4-6 nurses. 114 family physicians and 97 nurses participated to our study on voluntary basis working at 39 family health centers in urban Malatya. Primary care services are conducted by general practitioners or family physicians who allowed practicing at the family health center after receiving a certificate on family medicine adaptation course after six years of basic medical education and family medicine specialists; who complete minimum three years of vocational training in Turkey. Family medicine specialists serving in the field are less in number therefore they are not included in this study. Nurses also could work after completing 2-4 years of basic nurse colleges.

The Survey Form

All the questions were prepared after the literature search. Survey form consisted of three main items. The initial part of the questionnaire was about the "professional identity information" and demographic characteristic of primary care physician and nurses. Second part consisted of ten questions about the knowledge and attitude of evaluating the sufficiency of breast milk of mothers. In the third part the participants were asked nine questions regarding the management in case of the inadequacy of breast milk. 5 point Likert scale questionnaire were used responses were recorded as "strongly agree", "agree", "neutral", "disagree", "strongly disagree". The survey form was circulated and collected by intern students at the rotation of family medicine department at Inonu University School of Medicine. Each participant filled out the questioner form by herself / himself.

Statistics

SPSS for windows version 16.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Results were expressed as mean and minimum and maximum values. Descriptive statistics and chi-square, analysis of variance with statistical significance set at P < 0.05.

Results

A total of 139 physicians and 139 nurses were asked to participate in this study. 18 physicians and 33 nurses did not accept to fill out the form. Seven physicians and nine nurses were excluded from the study due to the missing data. In total, 114 physicians and 97 nurses completed the study Participants were asked to respond all the questions. The characteristics of the 211 health care providers in the study are shown in Table 1.

82.4% of physicians and nurses participating in our study were serving in the primary care over ten years. The percentage of physician and nurses who never seen a case of neonatal hypernatremic dehydration was 87.7% and 96.9% respectively. 14.1% of physician and 7.2% of the nurses had not studied theoretically Hypernatremik dehydration during their professional education. 73.7% percentage of physician stated that they weigh infants in every encounter; this application was relatively lower, 47.4% in nurses. 55.7% of the nurses and 59.6% of the physician were of the opinion that 10% or more loss of the weight from birth weight indicates the inadequacy of breast milk Table 2.

78.9% of the physicians and 64.9% of the nurses think that NHD may cause intracranial and renal failure problems. Therefore referral of these cases to the secondary and tertiary care centers would be suitable Table 3.

The confidence of nurses and physician in the management of NHD in the primary care were 12.4% and 18.4% respectively (Table 3). Physicians were suggesting to continue breastfeeding to the mother explaining the amount of milk will increase as she keeps on breastfeeding at the rate of 78.1%.

57% of physicians and 63.9% of nurses think that NHD may be the result of insisting on breas-tfeeding when breast milk is insufficient.

61.4% of the physicians and 84.5% of the nurses either have no opinion or disagree to initiation infant formula when breast milk insufficient (Table 3).
Sociodem featu	ograp ires	hic	NH	D educa %	ation	Eve	r Seen case %	NHD	Wei	Weighing Habi %	
No		%	Yes	No	p *	Yes	No	р	Yes	No	р
					Gen	der		-			
Male	115	54.5	12.2	83.5	<0.05	8.7	91.3	>0.05	68.7	31.3	<0.05
Female			7.3	92.7		7.3	92.7	0.00	53.1	46.9	10.00
Age											
20-29	11	5.2	0	100		0	100		81.8	18.2	
30-39	111	52.6	9.9	90.1	>0.05	9.0	91.0	>0.05	64.9	35.1	>0.05
40-49	89	42.2	11.2	83.1		7.8	92.2		55.1	44.9	
					Occup	ation					
Doctor	114	54.0	14.1	85.9	-0.05	12.3	87.7	-0.05	73.7	26.3	<0.01
Nurse	97	46.0	7.2	92.8	< 0.05	3.1	96.9	<0.05	47.4	52.6	<0.01
					Marital	Status					
Married	191	90.5	11.5	88.5	>0.05	7.3	92.7	>0.05	61.9	38.8	>0.05
Single	20	9.5	5.0	95.0	-0.05	15.0	85.0	-0.05	65.0	35.0	20.05
				S	ervice I	Duration	n				
0-4 years	13	6.2	0	100.0		0	100		53.8	46.2	
5-9 years	22	10.4	27.3	72.7	>0.05	40.9	59.1	< 0.01	68.2	31.8	>0.05
≥ 10 years	176	824	9.6	90.4		4.5	95.5		61.4	38.6	

Table 1. Sociodemographic features, weighing habit and awareness of NHD

Table 2.	Primarv	care workers	knowledge i	n the a	assessment	of breast	milk sui	fficiencv	(n:211))
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	Responses (%)				
Statements		0	Occupation		
		Doctor	Nurse	р	
Paby's weight is important to determine the	Agree	22.8	17.5		
baby s weight is important to determine the	No opinion	45.6	37.1	>0.05	
adequacy of breast mink	Disagree	31.6	45.4		
Project fullness is an important parameter to	Agree	22.8	17.5		
understand the sufficiency of breast mills	No opinion	45.6	38.1	>0.05	
understand the sufficiency of breast mink	Disagree	31.6	44.3		
Proast mills production indicator adoquate	Agree	24.6	40.2		
breast mills	No opinion	17.5	23.7	< 0.05	
oreast mirk	Disagree	57.9	36.1		
10% or more loss of the weight from birth	Agree	59.6	55.7	>0.05	
weight indicates the inadequacy of breast	No opinion	30.7	25.8		
milk	Disagree	9.7	18.5		
Calm and pagaaful baby after suchling shows	Agree	40.4	42.3		
the adequacy of the broast mills	No opinion	50.0	33.0	< 0.05	
the adequacy of the bleast mink	Disagree	9.6	24.7		
To see the milk in the mouth of the baby	Agree	30.7	16.5		
during the suckling and swallowing sound	No opinion	38.6	36.1	<0.05	
makes me think about the adequacy of the breast milk	Disagree	30.7	47.4		
There is a relationship between the number	Agree	85.1	85.1 51.5		
of urination of baby per day and adequacy of	No opinion	8.8	22.7	< 0.05	
breast milk	Disagree	6.1	25.8		

Appearance of urate crystals (pink color	Agree	20.2	13.4	
formation) in the urine indicates sufficiency	No opinion	14.9	5.2	< 0.05
of breast milk	Disagree	64.9	81.4	
Fever during the first week of life may be the	Agree	20.2	13.4	
cause of feeding problems	No opinion	52.6	55.2	< 0.05
	Disagree	27.2	81.4	
Breast milk is very low The level of sodium	Agree	75.4	37.1	
in blood may increase if the infant is fed	No opinion	12.3	32.0	< 0.05
inadequately	Disagree	12.2	30.9	

		Doctor	Nurse
NHD may be the result of insisting on breastfeeding when	Agree	43.0	36.1
hreast wills is insuff signt	No opinion	29.8	33.0
breast milk is insufficient	Disagree	Doctor N 43.0 3 29.8 3 27.2 3 78.1 5 12.3 1 9.6 2 57.9 3 26.3 1 15.8 4 38.6 1 14.9 2 46.5 5 57.9 3 8.8 1 33.3 4 86.0 7 5.3 8 8.7 1 20.2 1 32.5 7 47.3 7 18.4 1 14.0 1 67.6 7 78.9 6 14.1 2	30.9
I suggest to continue breastfeeding to the mother	Agree	78.1	56.7
explaining the amount of milk will increase as she keeps on	No opinion	12.3	19.6
breastfeeding	Disagree	9.6	23.7
I suggest giving additional water while continued	Agree	57.9	37.1
hrongtfonding	No opinion	26.3	13.3
Uleastieeunig	Disagree	15.8	49.5
I suggest giving infant formula in addition to continued	Agree	38.6	15.5
hreastfeeding	No opinion	14.9	28.9
breastreeding	Disagree	46.5	55.6
I suggest giving additional rice flour in addition to	Agree	57.9	37.1
breastfeeding	No opinion	8.8	13.4
	Disagree	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	49.5
I recommend drinking at least 2.5 liters of water a day for	Agree	86.0	74.2
mothers	No opinion	5.3	8.3
	No opinion 29.8 33 Disagree 27.2 30 Agree 78.1 56 No opinion 12.3 19 Disagree 9.6 23 Agree 57.9 37 No opinion 26.3 13 Disagree 15.8 49 Agree 38.6 15 No opinion 14.9 28 Disagree 46.5 55 Agree 57.9 37 No opinion 14.9 28 Disagree 46.5 55 Agree 57.9 37 No opinion 8.8 13 Disagree 33.3 49 Agree 86.0 74 No opinion 5.3 8. Disagree 8.7 17 Agree 20.2 13 No opinion 32.5 7 Disagree 47.3 79 Agree 18.4	17.5	
Lencourage mothers to eat more sugary foods and drinking	Agree	20.2	13.4
the water	No opinion	32.5	7.2
	Disagree	47.3	79.4
	Agree	18.4	12.4
I think I can manage NHD in primary care set up.	No opinion	14.0	16.5
	Disagree	67.6	71.1
NHD may cause intracranial and renal failure problems.	Agree	78.9	62.9
Therefore I refer this cases to the secondary and tertiary	No opinion	14.1	23.7
care centers	Disagree	7.0	13.4

Table 3. Primary care providers management in breast milk insufficiency Statements

Discussion

Although NHD's actual incidence is not known; however studies indicate an increase in the frequency and severity of this condition (9). Probability of early detection and intervention of NHD by the primary care physicians and nurses is theoretically seems to be possible. To the best of our knowledge this is the first study focusing on the awareness of the primary care healthcare givers concerning NHD. In this study we found insufficient knowledge, awareness and attitude concerning breastfeeding and neonatal hypernatremic dehydration in the primary care healthcare givers. Our study population comes across rather low rates of NHD. This data contradicting with the literature could be interpreted as missing out of actual NHD cases in primary care. Some hospital based studies provoked awareness over past few years (10). Unfortunately there are no data in the literature regarding the incidence of NHD in primary care set up. Both physicians and nurses had not been instructed theoretical and practical education on the topic of NHD throughout their training. This could be due to the participant's long period of graduation from their medical faculty, nursing school. Relatively new graduates might have some degree of raised awareness on the subject owing to available literature (11). Participants of our study have the habit of weighing babies at each examination. The interpretation of the weight of the infant in context to early detection of NHD falls short of the mark (12). In a study conducted by Konetzny et al. in Switzerland; weighing infants everyday ensuing the day of birth is an easy and cost-effective technique to identify dehydration in neonates (13).Similarly Iyer et al. indicated the importance of weighing as early as 72 hours. In addition support of breastfeeding along with close follow up of weighing may decrease intensity of NHD and allow mothers to carry out breastfeeding in the necessity of NHD related hospitalization (14). Emphasis on the meaning of daily weighing should be promoted by continuous education at the primary care. Newborns admitting to the primary care health centers should be evaluated in terms of weight below the expected ideal weights.

Questions to identify sufficiency of breast milk like, fever during the first week of life may be the cause of feeding problems, appearance urate crystals (pink color formation) in the urine indicates sufficiency of breast milk, to see the milk in the mouth of the baby during the suckling and swallowing sound makes me think about the adequacy of the breast milk has not been responded correctly by the majority of the participants. This information could easily be obtained during the infant's examination or from the history taken from the mother. These questions are basic clues and harvesting of an imminent NHD. Hereby health care provider enables to determine next most appropriate step. Much importance has been given to the initiation, duration of or increased rate of breastfeeding or beneficial effects of breast milk on baby as well as on mother in the literature over decades. On the other hand the effect of mother's education and support on the success rate of breast feeding is also a matter of concern to some researches(15). However data is limited for primary carebased interventions originating from a clinician's office (16). A study conducted Brodribb et al. had consistent findings with our study in context the lacked breastfeeding knowledge for the primary care registrars. Hence primary care practitioner's adequate knowledge and skills are critical to be able to guide and support breastfeeding women (17). Family physicians and primary health care units are first contact to all kind of patients. Also they are available round the clock. Management of NHD could not be possible due to varying facilities of different primary care units in different countries. Prolonged stay at hospitals just for the purpose of weight follow up may associate with hospital related complications. Unavailability or uncertainty of proper follow up of newborns may delay hospital discharge. One solution to this dilemma is to cooperation of tertiary care with primary care in continuous medical education and close follows up of infants in the first week of discharge from the hospitals (18). Lock et al. offered prolongation of hospital stays after the deliveries to prevent the occurrence of NHD (19). This approach would increase the hospital related complications and expenses rather preventing NHD. Instead increasing awareness and facilities in primary care would appear to be easy, applicable and cost effective method in early diagnosis and intervention to NHD cases. Majority of the physicians and nurses in our study have a general unwillingness suggest supplemental infant formula to the babies with breastfeeding problems. Beneficial effects of breast milk are known (20). Nevertheless we found unnecessary reluctance to suggest infant formula when required. Insisting on breast milk when it is totally absent that is; inadequate transfer of breast milk from mother to infant may cause NHD. Education of physicians on different issues yielded improved outcomes in primary care (21). Physicians and nurses in this study stated low degree of confidence in the management of NHD in primary care. This could be due current healthcare system reform which brings new legislation and uncertainties in the working conditions. What we believe is; as many different preventable diseases NHD also could be managed successfully in the primary care. Because "primary care helps prevent illness and death" (22)

Conclusion

This study remarks incomplete knowledge, awareness and attitude among primary care health care providers (nurse and physician) on NHD. We believe the emphasis on continuing medical education would contribute to decrease NHD related morbidity and mortalities.

Limitations and strength of the study

This trial is emphasizing the importance of early recognition of NHD in the primary care the trials concerning this issue is very limited in the literature. The results of this study could not be generalized as it has been performed in one city where the family medicine transformation has been implemented. The majority of the physicians were male limiting the views of female physicians.

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References

- Keister D, Roberts K, Werner L. Strategies for Breastfeeding Success. Am Fam Physician. 2008;78(2):225-32.
- American., Academy., of., Peadiatrics. Breastfeeding and the use of human milk. Work Group on Breastfeeding. 1997 Contract No.: 100.
- 3. Imdad A, Yakoup Y, Bhutta Z. Effect of breastfeeding promotion interventions on breastfeeding rates, with special focus on developing countries. BMC Public Health. 2011;13:24.
- 4. Soskolne E, Schumacher R, Fyock C. The effect of early discharge and other factors on readmission rates of newborns. . . Arch Pediatr Adolesc Med 1996;150:373-9.
- Manganaro R, Mami C, Marrone T, Marseglia L, Gemelli M. Incidence of dehydration and hypernatremia in exclusively breast-fed infants. J Pediatr. 2001;139:673-5.
- 6. Livingstone V, Willis C, Abdel Wareth L, Thiessen P, Lockitch G. Neonatal hypernatremic dehydration associated with breast-feeding malnutrition: a retrospective survey. CMAJ. 2000;162:647-52.
- Michael M, Manole D, Bogen D, Ayus C. Breastfeeding-Associated Hypernatremia: Are We Missing the Diagnosis. Pediatrics 2005;116(3):343-7.
- 8. Kaplan J, Siegler R, GA. S. Fatal hypernatremic dehydration in exclusively breast-fed newborn infants due to maternal lactation failure. . Am J Forensic Med Pathol. 1998;19:19-22.
- 9. Neifert M. Prevention of breastfeeding tragedies. Pediatr Clin North Am 2001;48(2):273-97.

- Uras N, Karadağ A, Dogan G, Tonbul A. Moderate hypernatremic dehydration in newborn infants: Retrospective evaluation of 64 cases. The Journal of Maternal-Fetal and Neonatal Medicine. 2007;20(6):449-52.
- 11. Laing I, Wong C. Hypernatraemia in the first few days: is the incidence rising? Arch Dis Child Fetal Neonatal Ed 2002;87:158-62.
- 12. Harding D, Cairns P, Gupta S, Cowan F. Hypernatraemia: why bother weighing breast fed babies? . Arch Dis Child Fetal Neonatal Ed. 2001.;85:145.
- 13. Konetzny G, Bucher H, Arlettaz R. Prevention of hypernatraemic dehydration in breastfed newborn infants by daily weighing. . Eur J Pediatr. 2009;168:815-8.
- 14. Iyer N, Srinivasan R, Evans K, Ward L, Cheung W, Matthes J. Impact of an early weighing policy on neonatal hypernatraemic dehydration and breast feeding. Arch Dis Child. 2008;93:297
- 15. Porteous R, Kaufman K, Rush J. The effect of individualized professional support on duration of breastfeeding: a randomized controlled trial. J Hum Lact. 2000;16(4):303-8.
- 16. Guise J, Palda V, Westhoff C, Chan B, Helfand M, Lieu T. The Effectiveness of Primary Care- Based Interventions to Promote Breastfeeding:Systematic Evidence Review and Meta-Analysis for the US Preventive Services Task Force. Annals of Family Medicine. 2003;1(2):70-8.
- 17. Brodribb W, Fallon A, Jackson C, Hegney D. Breastfeeding and Australian GP Registrars—Their Knowledge and Attitudes. J Hum Lact 2008;24:422-30.
- Chilton L. Prevention and management of hypernatremic dehydration in breast-fed infants. West J Med. 1995;163(1): 74–76. (1):74-6.
- 19. Lock M, JG R. Higher neonatal morbidity after routine early hospital discharge: are we sending newborns home too early? CMAJ. 1999;161:249-52.
- 20. Riordan M. The cost of not breastfeeding: a commentary. J Hum Lact. 1997;13(3):93-7.
- Mazmanian P, Davis D, Galbraith R. Continuing Medical Education Effect on Clinical Outcomes: Effectiveness of Continuing Medical Education: American College of Chest Physicians Evidence-Based Educational Guidelines. Chest. 2009;135 (3):49-55.
- 22. Starfield B, Letyu S, Macinko J. Contribution of Primary Care to Health Systems and Health. The Milbank Quarterly. 2005;83(3):457-502.

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The degree of conversion of orthodontic adhesives polymerized with visible light and toxicity of their eluates

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Abstract

Introduction: Polymer-based materials used in dentistry demonstrate potentially harmful biological effects in laboratory conditions.

Objectives: The aim of this study was to assess the degree of conversion (DC) of orthodontic adhesives after polymerization with visible light and their eluates' cytotoxicity in in vitro conditions.

Material and methods: The study assessed the DC of six orthodontic adhesives, Contex LC (Dentaurum), Resilence (Ortho Technology), Light Bond (Reliance), TransBond XT (3M), Green Gloo (Ormco) and Aspire (Ortho Classic), polymerized with LED light with the use of the Fournier transform infrared spectroscopy (FTIR). In order to evaluate the cytotoxicity of eluates of studied materials, MTT assay was carried out in cultured 3T3 fibroblast cells. The obtained results were analyzed statistically at the significance level of p = 0.05, assessing - among other things – the correlation between the DC of the material and the cytotoxic effects of its eluates in in vitro conditions.

Results: The DC of orthodontic adhesives assessed 24 hours after polymerization of samples ranged from 33.23% in the case of the Light Bond to 79.66% for the Aspire. A statistical analysis revealed no correlation between the DC of the materials and the level of cytotoxicity of their eluates.

Conclusions: 1. Under the conditions of the present study, the cross-linking of orthodontic adhesives is not complete and depends on the type of material. 2. Under the conditions of the present study, the cytotoxicity of eluates of orthodontic adhesives depends on the type of material, and not on the degree of its conversion.

Key words: Dental composites, orthodontic adhesives, degree of conversion.

Introduction

During the last decades we have been observing tremendous progress in the development of dental materials group, whose composition is based on polymers. Composite materials applied in conservative dentistry are used for reconstruction of damaged hard tissues of teeth, in prosthetics for implementation of permanent and temporary restorations to missing teeth or cementing prosthetic restorations, and in orthodontics in the form of adhesive resins they are used for fixing brackets on the enamel surface. Both research teams and the medical industry representatives are mainly focused on improving their physical properties and usability, as well as through introduction of new monomer molecules to production ^{1,2,3} they are trying to minimize the effect of polymerization shrinkage of materials used for fillings, responsible for development of secondary caries ^{4,5,6}.

Composite materials used in dentistry, including polymer-based orthodontic adhesives, are made of organic matrix, inorganic fillers, initiators, catalysts and inhibitors of the polymerization reaction. While the inorganic component of orthodontic adhesive resins is responsible for their positive characteristics, such as resistance to mechanical stimuli, the organic polymer network seems to be less than perfect component of the materials. The composite material matrix is made of monomers such as Bis-GMA and TEGDMA and comonomers responsible among other things for their plasticity, among which the most popular are UDMA, EDGMA and HEMA 7,8,9. During the polymerization process, monomer molecules are linked together to form a spatial polymer network with a fraction of inorganic fillers. Polymerization of most currently used orthodontic adhesives

is initiated by visible light emitted by the operating lamp. The use of such a solution is supported by convenient application in a clinical setting and control of the curing process. Unfortunately, studies conducted in many centers indicate that the cross-linking process of composite materials used in dentistry is incomplete both in the case of materials polymerized with light and through a chemical reaction ¹⁰. As a result of incomplete polymerization of the composite material, partially unbounded monomers remain in its mass, which may be released to the outside environment 11. Incompletely polymerized composite material of non homogenous structure is more susceptible to degradation under the influence of physical and chemical factors ¹²⁻¹⁶. In clinical conditions this phenomenon is manifested by weight loss of the material, weakening of the adhesion to the tooth tissues, and susceptibility to staining ¹⁷.

Studies conducted in laboratory conditions, and few experiments carried out in in vivo conditions indicated that polymer-based dental materials are chemically unstable and release their components to the external environment 18,19,20,21. So far about 30 22 components released from dental materials have been identified, including biologically harmful monomers such as TEGDMA, UDMA, EDGMA, HEMA or bisphenol A. Monomers released to the external environment have cytotoxic ^{23,24,25,26}, mutagenic properties ^{27,28,29,30} and can be potent allergens ^{6,31,32,33}. Bisphenol A released from dental materials 34,35, which is a component of Bis-GMA resin, also demonstrates parahormonal activity 36,37 through the activation of hormone receptors of the estrogen group as well as neurotoxic activity 38,39. Experiments with laboratory animals indicated that BPA causes damage to developing embryos, impairment of female fertility and spermatogenesis in males and it induces changes in behavior through acting on the developing nervous system ^{38,40,41,42}. The release of potentially harmful chemicals from dental materials occurs in two mechanisms. In the initial phase after the application of the polymerized material, components unbounded with the polymer network are released, at a later time the release of components is associated with degradation of the material under the influence of physical and chemical factors ^{43,44,45}. There is no doubt that a lower degree of polymer conversion is associated with the presence of greater amounts of free monomers in its mass, thus with release of potentially biologically dangerous chemicals to the external environment. An ideal dental material should be converted 100% into a stable and solid structure in the polymerization process. The degree of cross-linking of composite materials polymerized with light depends on many factors, including: the chemical structure of monomers, photoinitiator effectiveness, the type of filler, the translucency of the material, thickness of the cured layer, the distance from the light source to the material, light intensity and its emission time as well as the composition of the atmosphere where process takes place ^{17,19,46,47,48}. During clinical work it is often not possible to ensure optimum conditions for the polymerization process. Difficult access to cavities often prevents proper positioning of the curing light during irradiation of the material. Polymerization of orthodontic adhesives beneath the bases of brackets results in the light's limited access to the mass of the material. The presence of air, and thus oxygen in the oral cavity contributes to disturbances of polymerization of composite materials and to formation of the so-called oxygen inhibition layer on their surface, in which the polymerization does not occur or occurs to a small extent ^{19,49}. Laboratory tests often create optimal conditions for the polymerization process, therefore it seems that in such tests the degrees of conversion of dental materials may be higher than those achieved in clinical conditions. All medical products which are drugs or materials in direct contact with the tissue should meet the condition of not having cytotoxic properties. This requirement is in accordance with existing EU law ⁵⁰, which requires manufacturers to test medical devices for tissue compatibility.

The choice of a method for assessing cytotoxicity of a drug or a chemical compound depends on the type of preparation, the nature of its activity, the tissue distribution and the method of elimination from the body. Many aspects of chemical compounds' activity can be satisfactorily observed in tissue cultures. In laboratory practice, there are many methods for testing the cytotoxicity, which may be performed in in vitro conditions, assessing the status of the cells treated with potentially harmful substances. In order to assess the biologi-

cal impact of dental materials, gingival fibroblasts, keratinocytes of oral epithelium and standardized strains of L-929 and 3T3 mouse fibroblasts are commonly used ^{51,52}. In the case of medical products' cytotoxicity, a method for evaluating the impact of eluates from tested materials on the cells was also used. This method allows to imitate oral cavity conditions where dental composite materials are in constant contact with saliva or fluids consumed by patients. Available literature provides information on the use of a variety of tests for cytotoxic activity of dental materials and their ability to induce cell dysfunction, to express specific proteins and finally to induce cell death. They include: tests assessing the amount and damage of DNA 53, a test assessing the amount of glutathione ^{54,55}, a test evaluating the expression of heat shock proteins 56 and examinations evaluating the severity of apoptotic activity 57,58,59. Each of these methods has advantages and disadvantages, and provides additional information that is not obtained in other research. The most commonly used methods to assess the cytotoxic activity of dental materials in tissue culture is the MTT assay / assay of succinate dehydrogenase activity 60,61,62, which uses the capacity contained in the mitochondrial succinate dehydrogenase of viable cells to catalyze the reduction reaction of the thiazolyl blue formazan to water-insoluble formazan. The reduced compound has red color, and its concentration in the solution expressed by color change is evaluated with the use of a spectrophotometer. The more intense the color change is observed in the solution, the more viable cells are in culture. A comparison with control material allows the assessment of cytotoxic activity of a potentially harmful chemical compound.

Aim of the Study

The aim of this study was a comparative assessment of the degree of conversion in light polyme-*Table 1. Orthodontic adhesive resins assessed in the present study*

rized orthodontic adhesives and assessment of cytotoxicity of eluates obtained after storage of evaluated materials' samples in water.

Materials and Methods

Materials

Six orthodontic adhesives widely used in Poland were assessed: Contex LC (Dentaurum, Germany), Resilence (Ortho Technology, USA), Light Bond (Reliance Orthodontic Products, USA), TransBond XT (3M, USA), Green Gloo (Ormco, USA), Aspire (Ortho Classic, USA). The assessed orthodontic adhesive systems came from Polish distribution sources, and were authorized for sale on the EU market. The evaluated materials are shown in Table 1.

The Evaluation of the Conversion In Orthodontic Adhesives

In this study the extent of conversion of orthodontic adhesive samples (listed in Table 1) after polymerization carried out with the use of the 55 LED Curing Light (TPC Advanced Technology, USA) was evaluated. In order to measure the degree of conversion in the assessed orthodontic adhesive resins and composite materials the method of Fournier transform infrared spectroscopy was used. A FTIR type spectrometer (Fourier Transform Infrared Radiation) was used, namely Nicolet IS 10 from Thermo Scientific (USA) with a Smart Orbit accessory with a diamond crystal (type IIa). The device used in this study is presented in Figure 1. In order to obtain uniform sample volumes, the studied materials were placed in Teflon matrices 5 mm in diameter and 2 mm in depth. Then they were removed them from the matrices and transferred onto the diamond crystal of the Smart Orbit accessory. Subsequently, the spectrum of infrared radiation reflected from the test materi-

trade name	producer	country of origin	batch	sell-by date
Contex LC	Dentaurum	Germany	REF 163-511-00	2012-09
Resilence	Ortho Technology	USA	B 1711-1	2013-08
Light Bond	Reliance Orthodontic Products	USA	111995	2013-09
TransBond XT	3M	USA	N 222173	2013-11
GreenGloo	Ormco	USA	3665125	2013-03
Aspire	Ortho Classic	USA	EG26M	2012-08

al in 32 repetitions was recorded in order to obtain the distribution in the wave number range from 4000 to 400 cm⁻¹. After recording the spectrum, the material was removed from the accessory crystal, placed between two layers of polypropylene film and formed with the use of Teflon plates to obtain a layer 1 mm thick. After removing the Teflon plates, the assessed material was polymerized with the LED 55 Curing Light from TPC Advanced Technology Company (USA) for 20 seconds. The distance of the light source from the sample surface was 5 mm. After completion of the polymerization process described above, the material was removed from between the polypropylene films and was left for one hour at 25°C. The polymerized samples were transferred again onto the diamond crystal of a Smart Orbit accessory and then the spectrum of infrared radiation reflected from the material was analyzed. Subsequently, the studied materials were placed in separate containers, and the samples were analyzed again with the use of the described above method after 24 hours, 7 days and 30 days of storage.

The records of infrared spectra obtained with the measurement described above were used to assess the degree of conversion of orthodontic adhesive systems in four time intervals, i.e. after 1 hour, 24 hours, 7 days and 30 days from the initiation of polymerization. The degree of conversion / DC of the assessed dental materials was calculated using the formula ^{2,63,64,65,66}:



Figure 1. IS10 Nicolet spectrometer (Thermo Scientific, USA) with a Smart Orbit accessory

$$DC(\%)=[1 - \frac{(A C=C after/A C=O after)}{(A C=C before/A C=O before)}] \times 100$$

- A C=C after the area of the peak at the wavelength of 1638 cm-1 corresponding to the vibrations of the double bond of carbon = carbon after polymerization
- A C=O after the area of the peak at the wavelength of 1720 cm-1 corresponding to the vibration of the carbonyl group bonds after polymerization
- A C=C before the area of the peak at the wavelength of 1638 cm-1 corresponding to the vibrations of the double bond of carbon = carbon before polymerization
- A C=O before the area of the peak at the wavelength of 1720 cm-1 corresponding to the vibration of the carbonyl group bonds before polymerization

Cytotoxicity assessment in eluates of the studied orthodontic adhesives

In order to obtain dental material eluates, five samples of each of the assessed orthodontic adhesives were prepared. Polymers were placed in hollows of teflon matrixes 5 mm in diameter and 2 mm deep, and then each sample was polymerized with LED 55 Curing Light from TPC Advanced Technology (USA) for 20 seconds. The light intensity of more than 1,100 mW/cm² was confirmed by optical power measurement performed with a PM100 meter (Thornlabs, USA) with a thermal detector. The end of the device's fiber was at a distance of 5 mm from the polymerized material sample. After completion of the polymerization process, the samples were removed from the matrices with glass spatulas and placed in glass vials filled with 10 ml / 10 cm² HPLC grade water with 0.05 ml of Antibiotic Antimycotic preparation (Invitrogen, USA) containing amphotericin B, streptomycin and penicillin (in order to avoid microbial contamination of the solutions.) Such prepared samples were placed in C24 Classic incubator shaker (New Brunswick Scientific, USA) operating at the frequency of 112 cycles per minute. The temperature in the chamber of the device was 37°C, which corresponds to the temperature prevailing in the oral cavity. After the five samples of each of the studied materials were incubated in the conditions described above for 7 days, the materials were removed from the test tubes, and the obtained aqueous eluates were used for cytotoxicity assessment of composite materials. Cytotoxicity evaluation of the studied materials was conducted with assay of succinate dehydrogenase activity / MTT assay. Balb / c 3T3 mouse fibroblast cell line cells were plated in 96well Nunclon plates (Nunc, Denmark) at the density of 1x10⁵/ml (1x10⁴/well) in DMEM / Dulbeco Minimal Essential Medium (Sigma-Aldrich, USA) with 10% dialyzed fetal bovine serum / FBS / (Invitrogen, USA) and 1% Antibiotic Antimycotic poly-antibiotic mixture (Invitrogen, USA) at 100 µl / well. Culturing was carried out at 37°C in an atmosphere containing 5% CO₂, at 95% humidity. After 18 hours, cell morphology was assessed by microscopic observation. Then the medium was removed and the cell cultures were washed with PBS / phosphate buffered saline (Invitrogen, USA) at pH 7.4. The studied eluates from composite materials, buffered with 0.9% NaCl, were plated in 6 repetitions, with a control group of Antibiotic Antimycotic (Invitrogen, USA) poly-antibiotic mixture buffered in saline solution in HPLC grade water (Chromasolw for HPLC, Sigma Aldrich, USA) without the presence of studied material samples. Cells were incubated with eluates from the assessed composite materials for 60 minutes in culture conditions (see above), and then the plated solutions were gently removed from cells. The tiles were plated with DMEM / Dulbeco Minimal Essential Medium in the amount of 100 µl containing 50 µl solution of MTT / thiazolyl blue tetrazolium bromide (Sigma-Aldrich, USA) in PBS solution (Invitrogen, USA) thus obtaining the final concentration of 0.5 mg / ml of MTT. The studied 3T3 fibroblast cultures were incubated for a further 3.5 hours, and then the medium was removed, and the resulting formazan crystals were dissolved in 100 µl of DMSO / dimethyl sulfoxide (Sigma-Aldrich, USA) by placing them for 15 minutes in the shaker Shaker 358S (Elan, Poland) operating at 70 rev. / min. Then, using RC Mutliskan reader (Thermo Fisher Scientific, USA), the absorbance of light (OD) at the wavelength of 540 nm and reference wavelength of 650 nm was read.

The data were analyzed after subtraction of OD values read at 540 nm from OD values read at 650 nm. The number of cells that survived incubation with eluates of the studied dental materials (prepared by the methods described in the analytical section) was expressed in percentage with reference to the number of cells in the control cultures, for which the value of 100% was assumed.

Methods of statistical analysis

The results were statistically analyzed at the assumed significance level of p = 0.05.

For cont i nuous variables the following were calculated: the size, the arithmetic mean, standard deviation, median, minimum and maximum values.

The basic tool in the analysis of a medium was the model of one-way analysis of variance (ANO-VA one-way). Normal distribution was assumed. The homogeneity of variance was tested with the Brown-Forsythe test. For multiple testing, Tukey's test was used. In order to investigate the correlation between the degree of conversion of the assessed orthodontic adhesives and the cytotoxic activity of their eluates, the testing method proposed by Pearson was used.

Results

The degree of conversion of the assessed orthodontic adhesives

The degree of conversion of the studied orthodontic adhesives after one hour from initiation of the polymerization process ranged from 43.12% in the case of GreenGloo material to 72.12% in the case of LC Contex, averaging at 56.09%. Statistical analysis carried out at the significance level of p = 0.05 showed that Light Bond, TransBond XT and GreenGloo materials were cross-linked to a significantly lesser degree than Contex LC, Resilence and Aspire adhesives.

After 24 hours from the commencement of the study, the conversion of the samples ranged from 33.23% for the LightBond material to 79.66% for the Aspire material, with the average value of 59.29%. Analysis of the results showed that LightBond Green Gloo materials were cross-linked to a significantly lesser degree (p <0.05) than the

other studied orthodontic adhesive systems. The degree of cross-linking of polymers evaluated after 7 days from the initiation of the polymerization process was from 33.62% for the Light Bond samples to 82.18% for the Aspire samples, averaging at 58.06%. Statistical analysis carried out at the level of p = 0.05 indicated that LightBond and GreenGloo adhesive resins were, as in the previously described observation period, cross-linked to a significantly lesser degree than the other tested materials. After 30 days of observation, the average degree of conversion of the evaluated orthodontic adhesives was 64.42%, ranging from 37.40% in the case of the GreenGloo material to 84.41% for the Aspire resin. Light Bond and Green Gloo adhesives still demonstrated significantly lower (p <0.05) degree of cross-linking in comparison to other materials.Statistical analysis of arithmetic averages describing the conversion of all assessed orthodontic adhesives indicated that average cross-linking of materials did not change significantly (p > 0.05) during particular periods of observation.

The assessment of the degree of cross-linking in the various materials with reference to observation periods indicated that the degree of conversion in Resilence, LightBond and Green Gloo adhesives, did not change significantly (p > 0.05) in time.

In the case of TransBond XT and Aspire adhesive systems, the cross-linking increased significantly (p < 0.05) after the first observation period and remained at a similar level (p < 0.05) after 24 hours, 7 days and 30 days from the commencement of the experiment.The degree of conversion of the Contex LC adhesive increased significantly (p < 0.05) on day 30 in the experiment, compared to previous observation periods. The degree of conversion in the evaluated orthodontic adhesives in various time intervals is shown in Table 2.

Cytotoxicity of eluates from assessed orthodontic adhesives

The average lifespan of 3T3 fibroblasts exposed to eluates of the evaluated orthodontic adhesives obtained after 1 hour of sample storage in water was 100.4%, and was not significantly different (p> 0.05) from the viability of the cells exposed to control solutions (100%). Only in the cultures treated with Aspire adhesive eluates, a significant decrease (p <0.05) of the level of metabolic processes to the value of 86.2%.was observed,

	mean degree of conversion (%) after									
adhesive	1 hour		24 h	4 hours 7 d		ays	30	days		
	%	std	%	std	%	std	%	std		
Contex CL	72,12	3,17	72,34	1,55	73,88	1,50	81,66	3,59		
Resilence	63,99	4,16	63,33	3,70	50,36	12,46	58,50	6,78		
Light Bond	46,06	20,96	33,23	15,11	33,62	4,47	45,85	14,17		
TransBondXT	44,89	8,01	70,15	5,70	72,22	1,02	73,79	3,82		
Green Gloo	43,12	3,68	41,94	10,51	34,58	16,33	37,40	12,90		
Aspire	68,39	2,30	79,66	3,22	82,18	4,07	84,41	4,50		
mean	56,09	14,87	59,29	18,89	58,06	21,41	64,42	19,90		

Table 2. The mean degree of conversion of the evaluated orthodontic adhesives in various time intervals

Table 3. The numerical values describing the intensity of the metabolic processes in cells treated with orthodontic adhesive eluates obtained in different periods of observation comparing to 100% of control

	mean intensity of the metabolic processes of 3T3 cells										
adhesive	1 hour eluates		24 hour	rs eluates 7 days		eluates	30 days eluates				
	%	std	%	std	%	std	%	std			
Contex CL	113,1%	20,7	112,7%	19,9	95,3%	11,2	106,8%	16,8			
Resilence	101,9%	7,7	90,7%	7,8	81,7%	12,9	107,9%	8,2			
Light Bond	88,6%	9,8	81,5%	5,5	76,6%	6,1	78,4%	6,9			
TransBondXT	103,9%	11,0	90,7%	7,2	89,7%	11,1	97,9%	6,5			
Green Gloo	108,6%	5,3	122,0%	14,0	140,6%	18,5	133,2%	15,3			
Aspire	86,2%	4,5	72,5%	3,5	79,1%	3,3	78,6%	5,2			
mean	100,4%	14,4	95,0%	20,2	93,9%	24,6	100,5%	21,6			

compared to control cultures. Eluates obtained after 24 hours of storage of Aspire, Light Bond and TransBond XT samples, reduced the viability of cell cultures significantly stronger (p < 0.05) than the control solution, to the levels of 72.5%, 81.5% and 90.7% respectively. Mean metabolic activity of 3T3 fibroblasts treated with the solutions obtained after 24 hours of observation was maintained at the level of 95%, not differing significantly from the values observed in control cultures.

The average metabolic activity of cells exposed to solutions obtained after 7 days of incubation of orthodontic adhesives' samples in water was 93.9%, not differing significantly (p> 0.05) from the level observed in the control group. Eluates of Light Bond, Aspire and Resilence adhesives significantly (p <0.05) reduced the activity of succinate dehydrogenase in fibroblasts compared to the control solutions. The metabolic activity of the cells treated with eluates of the above mentioned materials was 76.6%, 79.1% and 81.7% respectively.

The evaluation of solutions obtained after 30 days of observation revealed that the average metabolic activity in the cultures was 100.5%, and it was not significantly different (p > 0.05) from that observed in the control group, however a significant (p < 0.05) reduction in metabolic processes was observed in the case of fibroblasts exposed to Light Bond (78.4%) and Aspire (78.6%) resin eluates.

The numerical values describing the intensity of the metabolic processes in cells treated with orthodontic adhesive eluates obtained in different periods of observation are shown in Table 3.

The assessment of correlation between the degree of conversion of orthodontic adhesives and their eluates' impact on metabolic processes in 3T3 fibroblasts

The analysis of the results carried out using the Pearson method showed a positive correlation (-0.149) between the degree of cross-linking of the evaluated orthodontic adhesives and the cytotoxic effect of their eluates on metabolic processes of cells in tissue cultures.

Discussion

The research results obtained by various authors ^{2,65-70} describing the degree of conversion of composite materials for filling cavities, range from

32% to 70%. It should be noted that the described experiments were carried out in accordance with different polymerization protocols, sample preparation, measurement of the degree of cross-linking in materials and they related to different polymer systems.

Available literature reports few studies assessing the degree of conversion of orthodontic adhesives. In a study by Cerveira et al ⁷¹, the authors assessed the degree of conversion of the TransBond XT orthodontic adhesive using the FTIR method, which was also applied in the present study. Material samples were polymerized by light from diode and halogen lamp for 5, 10 and 15 seconds, and the quoted authors assessed the conversion of the material immediately after exposure. Depending on the curing lamp type used by Cerveira et al ⁷¹ and exposure time to the emitted light, the TransBond XT adhesive's degree of conversion ranged from 38.97% to 47.24%. In the present study, the degree of conversion of the TransBond XT material was 44.89% after 1 hour from polymerization of samples, 70.15% after 24 hours from polymerization of samples, 72.22% after one week from polymerization of samples and 73.79% after one month from polymerization of samples. The statistical analysis of the results of the study showed that the degree of cross-linking of the adhesive evaluated at the initial stage of observation was significantly lower (p < 0.05) than that recorded in subsequent periods. The results of the present study describing the degree of conversion of the TransBond XT material 1 hour after curing are similar to those obtained by Carveira et al 71 who evaluated the material immediately after polymerization. However, the increase of cross-linking in the TransBond XT adhesive in subsequent observation periods, which was observed in the present study, suggests that the polymerization process of at least some dental composites is a phenomenon that lasts even after the exposure of the material, until stabilization of its internal structure is obtained.

The method of Fournier transform infrared spectroscopy was also applied by Jagdish et al ⁷² who evaluated the degree of conversion of the TransBond XT resin polymerized with halogen light on bases of orthodontic brackets. According to the quoted authors ⁷², the degree of cross-lin-

king of the material after polymerization equaled 48.74%, which was similar to that noted in the present study during one-hour observation.

A higher, compared to the results observed by Cerveira et al ⁷¹ and Jagdish et al ⁷², degree of conversion of the TransBond XT material was reported Eliades et al⁷³. The quoted authors ⁷³ polymerized orthodontic adhesive applied onto bracket bases under anaerobic conditions. The authors used a halogen lamp, light emission continued for 20, 40 and 60 seconds. Eliades et al 73 assessed orthodontic adhesive cross-linking with the FTIR method. The authors ⁷³ observed that the conversion of the TransBond XT material was over 60% and did not change significantly with increasing exposure time of the samples. Although Eliadnes et al ⁷³ evaluated the degree of conversion of the TransBond XT material immediately after polymerization, the reported cross-linking of the TransBond XT material was higher than that observed in the present study after 1 hour of exposure, however it was lower than that noted in subsequent observation periods.

In the study by Gioka et al ⁷⁴, the authors investigated the degree of conversion of the Light Bond orthodontic adhesive, which is also evaluated in the present study. The material was polymerized on the bases of orthodontic brackets bonded to isolated human teeth. In order to assess the degree of resin's cross-linking, the quoted authors ⁷⁴ used the method of micro reflection analysis with Fournier transform infrared spectroscopy, and the evaluation was conducted immediately after polymerization of the material with a halogen lamp. Gioka et al ⁷⁴ reported the Light Bond adhesive's conversion at 47%. This result is very similar to that obtained in the present study after the one -hour observation, equaling 46.06%.

In the experiment conducted by Cerveira et al ⁷¹, the authors evaluated the TransBond XT resin polymerization process (3M, USA), performed with LED light for 15 seconds under anaerobic conditions. Using the FTIR method, also applied in the present study, the cited authors observed the degree of conversion of the polymer at the level of 47.24%. A result similar to that reported by Cerveira et al ⁷¹ was observed by Jagdish et al ⁷², who also assessed the degree of cross-linking of the TransBond XT material using the FTIR method, and according to them ⁷² its value was

48.74%. Jagdish et al⁷² used a halogen lamp in the polymerization protocol and the samples' exposure to radiation lasted 40 seconds. Unfortunately, the authors did not specify whether the cross-linking process in the orthodontic adhesive occurred in air access conditions and it did not change significantly with increasing exposure time of the samples.

The degree of cross-linking in the TransBond XT orthodontic adhesive resin was also evaluated in the present study using the FTIR method. Under the conditions of the experiment, it was 67.74% on average, which was similar to the result reported by Eliades et al ⁷³.

The stabilization (p < 0.05) of the degree of cross-linking in the TransBond XT adhesive observed in the present study after 24 hours, is confirmed by the results of experiments by Pithon et al ⁷⁵. The quoted authors assessed the degree of conversion of resin polymerized in Teflon matrices cured with LED light for 40 seconds after 24, 48, 72 and 168 hours. Pithon et al ⁷⁵ observed no significant differences between the degree of conversion in the samples in various time intervals. Both in this study and studies by other authors ^{17,75} significant differences were observed between the degree of cross-linking in individual orthodontic adhesives evaluated in subsequent time intervals after polymerization. However, the overall evaluation of the results of the study, and the experiments described by Corekci et al ¹⁷ and Pithon et al ⁷⁵, shows that the degree of conversion of orthodontic adhesives assessed 24 hours after polymerization appears to be a constant value, and its significant changes are observed only in the case of individual resins. The dynamics of the polymerization process of composite materials seems to depend on the type of resin, its chemical structure and it remains an individual feature. The results obtained by various authors on the degree of conversion of dental composites demonstrate large discrepancies. These differences may result from the type of polymer being evaluated, the applied methodology and testing equipment, photopolymerization protocol and method of sample preparation. There is no doubt that the polymers used in dentistry are converted to 100%, and the remaining free monomers in the material have an adverse effect on its durability and chemical stability.

The study did not demonstrate statistically significant (p = 0.05) differences in the cytotoxic effects of evaluated orthodontic adhesives' eluates, obtained after 1 hour of sample storage in water, on cell cultures. Only the solutions obtained from the storage of Green Gloo and Contex LC materials showed a statistically significantly lower (p =0.05) cytotoxic effect when compared to the other studied materials. A diversification in the degree of influence of eluates from the assessed materials on the metabolic processes in 3T3 fibroblasts was observed in the comparison of the evaluated composite materials' solutions collected after 24 hours, 7 days and 30 days of sample incubation in water. The analysis of the results of the MTT assay demonstrated that, in the case of solutions obtained in the three periods of sample storage, eluates from Aspire and Light Bond adhesives reduced the number of cells in the cultures most statistically significantly (p = 0.05) when compared with other evaluated solutions. Cytotoxic effect of orthodontic adhesives and their eluates on cell cultures was confirmed in the present study and it was described in publications by Jagdish et al ⁷², Gioka et al ⁷⁴ and Pithon et al ⁷⁵.

In the study by Jagdish et al ⁷², the authors analyzed the correlation between the degree of conversion of orthodontic adhesives and their cytotoxic effects in in vitro conditions. The quoted authors reported no significant correlation between the degree of cross-linking of the material and the intensity of metabolism in the cells of cultures, evaluated with the MTT assay. In the present study no correlation was observed between the degree of conversion of orthodontic adhesives and their cytotoxic effects on 3T3 fibroblasts cells. The lack of such correlation supports the idea that biologically harmful effects of dental materials based on monomers depends not only on the efficiency of the polymerization process, but also on their chemical composition and chemical compounds released into the environment

Summary

The results of the study and experiences described by other authors indicate that polymerization of orthodontic adhesives with light is not fully effective process leading to a 100% stable polymer network. This phenomenon, apart from mechanical and chemical degradation of materials in the environment of the oral cavity, is responsible for the emission to the outside environment of potentially harmful chemical compounds, whose cytotoxic effect was confirmed in numerous studies. Safe application of composite-based orthodontic adhesives requires further investigation in terms of the safety of their use in clinical conditions.

A comparison of published results of studies on subsequent generations of orthodontic adhesive resins allows the assessment of their chemical and physical properties, their biological effects, and consequently the safety of their application in the patient's oral cavity.

Conclusions

Under the conditions of the present study, the cross-linking of orthodontic adhesives is not complete and depends on the type of material.

Under the conditions of the present study, the cytotoxicity of eluates of orthodontic adhesives depends on the type of material, and not on the degree of its conversion.

References

- 1. Moon EJ, Lee JY, Kim CK, Cho BH. Dental restorative composites containing 2,2-bis[4-2-hydroxy-3-methacryloloxy propoxy) phenyl] propane derivatives and spiro orthocarbonates. J Biomed Mater Res, Part B: Appl Biomater. 2005; 73B: 338-346.
- 2. Jing F, Fang J, Haiping X, Bauhui J, Xiaoqing L. Properties of a new dental photocurable matrix resin with low shrinkage. J Wuhan Univ Technology- Mater Sci Ed. 2011; 26(2): 236-241.
- 3. Braga RR, Ferracane JL. Alternatives in polymerization contraction stress management. Crit Rev Oral Biol Med. 2004; 15: 176-184.
- 4. Silicas N, Eliades G, Watts DC. Light intensity effects on resin composite degree of conversion and schrinkage strain. Dent Mater. 2000; 16: 292-296.
- 5. Braga RR, Ballester RY, Ferracane JL. Factors involved in the development of polymerization shrinkage stress in resin composites: A systematic review. Dent Mater. 2005; 21: 962-970.
- 6. Drucker AM, Pratt MD. Acrylate Contact alergy: patient characteristics and evaluation of screening allergens. Dermatit. 2011; 22(2): 98-101.
- 7. Ferracane JL. Current trends in dental composites. Crit Rev Oral Biol Med. 1995; 6: 302-318.

- 8. Peutzfeld A. Resin components in dentistry: The monomer system. Eur J Oral Sci. 1997; 105: 96-116.
- 9. Rueggeberg FA. From vulcanite to vinyl, a history of resin in restorative dentistry. J Prosthet Dent. 2002; 87: 364-379.
- Moraes LGP, Rocha RSF, Menegazzo LM, de Araujo EB, Yakimitu K, Moraes JCS. Infrared spectroscopy: A tool for determination of the degree of conversion in dental composites. J Appl Oral Sci. 2008; 16(2): 145-149.
- 11. Goldberg M. In vitro and in vivo studies on the toxicity of dental resin components: a review. Clin Oral Invest. 2008; 12: 1-8.
- 12. Polydorou O, Trittler R, Hellwig E, Kummerer K. Elution of monomers from two conventional dental composite materials. Dent Mater. 2007; 23: 1535-1541.
- 13. Tabatabaee MH, Mahdavi H, Zandi S, Kharrazi MJ. HPLC analysis of eluted monomers from two composite resins cured with LED and halogen curing lights. J Biomed Mater Res. 2009; 88B: 191-196.
- 14. Bettencourt AF, Nevés ChB, de Almeida MS, Pinheiro LM, e'Oliveira SA, Lopes LP, Castro MF. Biodegradation of acrylic based resins: A rewiew. Dent Mater. 2010; 26: e171-e180.
- 15. Stoner BR, Piascik JR, Brown B, Wolter SD. A novel array chip to monitor in situ composite degradation using electrochemical impedance spectroscopy. Dent Mater. 2011; 27: 811-817.
- Andrysiak P, Marcinkowska A, Kubisz L, Hedzelek W. The response of acrylic materials to constant and impulse temperature stimulus. Protet Stomatol. 2010; 60(6): 493-500.
- Corekci B, Malkoc S, Ozturk B, Gunduz B, Toy E. Polymerization capacity of orthodontic composites analyzed by Fournier transform infrared spectroscopy. Am J Orthod Dentofacia Orthop. 2011; 139(4): e299-e304.
- 18. Ferracane JL. Hygroscopic and hydrolytic effects in dental polymer networks. Dent Mater. 2006; 22: 211-222.
- 19. Yap AU, Han VT, Soh MS, Siow KS. Elution of leachable components from composites after LED and halogen light irradiation. Oper Dent. 2004; 29: 448-453.
- 20. Sideridou ID, Achilias DS. Elution study of unreacted bis-GMA, TEGDMA, UDMA, and bis-EMA from light-cured dental resins and resin composites using HPLC. J Biomed Mater Res. 2005; 74B: 617-626.
- Tseng WY, Huang CH, Chen R, Lee MS, Chen J, Rueggeberg FA, Chen MH. Monomer conversion and cytotoxicity of dental composites irradiated with different modes of photoactivated curing. J Biomed Mater Res. 2007; 83B: 85-90.

- 22. Bakopoulou A, Papadopoulos T, Gerefis P. Molecural toxicology of substances released from resin based dental restorative materials. Int J Molec Sci. 2009; 10: 3861-3899.
- 23. Engelmann J, Janke V, Volk J, Leyhausen G, Neuhoff NV, Schlegelberger B, Geurtsen W. Effects of BisGMA on glutathione metabolism and apoptosis in human gingival fibroblasts in vitro. Biomater. 2004; 25: 4573-4580.
- 24. Chang M-Ch, Chen L-I, Chan Ch-P, Lee J-J, Wang T-M, Yang T-T, Lin P-S, Lin H-J, Chang H-H, Jeng J-H. The role of reactive oxygen species and hemeoxygenase-1 expression in the cytotoxicity, cell cycle alteration and apoptosis of dental pulp cells induced by Bis-GMA. Biomater. 2010; 31: 8164-8171.
- 25. Demicri M, Hiller K-A, Bosl C, Galler K, Schmalz G, Schweikl H. The induction of oxidative stress, cytotoxicity and genotoxicity by dental adhesives. Dent Mater. 2008; 24: 362-371.
- 26. Zhu T, Lim B-S, Park H-Ch, Son K.M, Yang H-Ch. Effects of the iron-chelating agent deferoxamine on triethylene glycol dimethacrylate, 2-hydroxylethyl methacrylate, hydrogen peroxide-induced cytotoxicity. J Biomed Mater Res B: Applied Biomater. 2012; 100B(1): 197-205.
- 27. Kleinsasser NH, Wallner BC, Harreus UA, Kleinjung T, Folwaczny M, Hickel R, Kehe K, Reichl FX. Genotoxicity and cytotoxicity of dental materials in human lymphocytes as assessed by the single cell microgel electrophoresis (comet) assay. J Dent. 2004; 32: 229-234.
- 28. Drozd K, Wysokinski D, Krupa R, Wozniak K. Bisphenol A-glycid methacrylate induces a broad spectrum of DNA damage in human lymphocytes. Arch Toxicol. 2011; 85: 1453-1461.
- 29. Yeh H-W, Chang M-Ch, Lin Ch-P, Tseng W-Y, Chang H-H, Wang T-M, Chen Y-J, Lin Ch-Ch, Yang T-T, Lin L-D, Jeng J-H. Comparative cytotoxicity of five current dentin bonding agents: Role of cell cycle deregulation. Acta Biomater. 2009; 5: 3404-3410.
- 30. Chang H-H, Chang M-Ch, Lin L-D, Lee J-J, Wang T-M, Huang Ch-H, Yang T-T, Lin H-J, Jeng J-H. The mechanism of cytotoxicity of urethane dimethacrylate to Chinese hamster ovary cells. Biomater. 2010; 31: 6917-6925.
- Goon A T-J, Bruze M, Zimerson E, Goh C-L, Koh D S-Q, Isaaksson M. Screening for acrylate/methacrylate allergy in the baseline series: uor experience in Sweden and Singapore. Contact Dermat. 2008; 59: 307-313.
- 32. Khamaysi Z, Bergman R, Weltfriend S. Positive patch test reactions to allergens of the dental series and the relation to the clinical presentation. Contatc Dermat. 2006; 55: 216-218.

- 33. Chen A Y-Y, Zirwas MJ. Denture Stomatitis. SKINmed. 2007; 2: 92-94.
- 34. Pulgar R, Olea-Serrano F, Novillo-Fertel A, Rivas A, Pazos P, Pedraza V, Navajas JM, Olea N. Determination of Bisphenol A and related aromatic compounds released from Bis-GMA-based composites and sealants by high performance liquid chromatography. Environ Health Perspect. 2000; 108(1): 21-27.
- 35. Eliades T, Voutsa D, Sifakakis I, Makou M, Katsaros Ch. Release of bisphenol A from light-cured adhesive bonded to lingual fixed retainers. Am J Orthod Dent Orthop. 2011; 139: 192-195.
- 36. Summary Report Joint FAO/WHO Expert Meeting to Review Toxicological and Health Aspects of Bisphenol A. 1-5th of November 2010, Ottawa, Canada. http://www.who.int/foodsafety/chem/chemicals/ BPA Summary2010.pdf
- 37. vom Saal F, Welhons WV. Large effects of small exposures. II. The importance of positive controls in low-dose research on bisphenol A. Environ Res. 2006; 100: 50-76.
- 38. Poimenova A, Markaki E, Rachiotis C, Kitraki E. Corticosterone-regulated actions in the rat brain are affected by perinatal exposure to low dose of bisphenol A. Neurosci. 2010; 167: 741-749.
- 39. Midoro-Horiuti T, Tiwari R, Watson SCh, Goldblum RM. Maternal Bisphenol A exposure promotes the development of experimental asthma in mouse pups. Environ Health Perspect. 2010; 118(2): 273-277.
- 40. Salian S, Doshi T, Vanage G. Neonatal exposure of male rats to bisphenol A impairs fertility and expression of sertoli cell junctional proteins in the testis. Toxicol. 2009; 265: 56-67.
- 41. Fernandez M, Bianchi M, Lux-Lantos V, Libertun C. Neonatal exposure to bisphenol A alters reproductive parameters and gonadotropin releasing hormone signaling in female rats. Environ Health Perspect. 2009; 117(5): 757-762.
- 42. Fernandez M, Bianchi M, Lux-Lantos V, Libertun C. Neonatal exposure to bisphenol A alters reproductive parameters and endocrine alterations resembling the polycystic ovarian syndrome in adult rats. Environ Health Perspect. 2010; 118(9): 1217-1222.
- 43. Hume WR, Gerzina TM. Bioavailability of components of resin based materials which are applied to teeth. Crit. Rev Oral Biol Med. 1996; 7: 172-179.
- 44. Geurtsen W. Biocompatibility of resin-modified filling materials. Crit Rev Oral Biol Med. 2000; 11: 333-355.
- 45. Ferracane JL. Hygroscopic and hydrolytic effects in dental polymer networks. Dent Mater. 2006; 22: 211-222.

- 46. Rueggeberg FA. State-of-the-art: Dental photocuring - A review. Dent Mater. 2011; 27: 39-52.
- 47. Sigusch BW, Völpel A, Braun I, Uhl A, Jandt KD. Influence of different light curing units on the cytotoxicity of various dental composites. Dent Mater. 2007; 23: 1342-1348.
- 48. Zhang Y, Xu J. Effect of immersion in various media on the sorption, solubility, elution of unreacted monomers and flexural properties of two model dental composite composition. J Mater Sci. 2008; 19: 2477-2483.
- 49. Franz A, König F, Lucas T, Watts DC, Schedle A. Cytotoxic effects of dental bonding substances as a function of degree of conversion. Dent Mater. 2009; 25: 232-239.
- 50. Huschek G, Hansen PD, Maurer HH, Krenzel D, Kayser A. Environmental risk assessment of medical products for human use according to European Commission recomendations. Environ Toxicol. 2004; 19(3): 226-240.
- 51. Drozd K, Wysokinski D, Krupa R, Wozniak K. Bisphenol A-glycid methacrylate induces a broad spectrum of DNA damage in human lymphocytes. Arch Toxicol. 2011; 85: 1453-1461.
- 52. Guess WL, Rosenbluth SA, Schmidt B, Autian J. Agar diffusion method for toxicity screening of plastics on cultured cells monolayers. J Pharm Sci. 1965; 54: 1545-1547.
- 53. Ansteinsson V, Solhaug A, Samuelsen TJ, Holme JA, Dahl JE. DNA-damage, cell cycle arrest and apoptosis induced in BEAS-2B cells by 2-hydroxymetyl methacrylate (HEMA). Mutation Res. 2011; 723: 158-164.
- Engelmann J, Janke V, Volk J, Leyhausen G, Neuhoff NV, Schlegelberger B, Geurtsen W. Effects of BisGMA on glutathione metabolism and apoptosis in human gingival fibroblasts in vitro. Biomater. 2004; 25: 4573-4580.
- 55. Chang H-H, Guo M-K, Kasten F.H, Chang M-Ch, Huang G-F, Wang Y-L, Wang R-S, Jeng J-H. Stimulation of glutathione depletion, ROS production and cell cycle arrest of dental pulp cells and gingival epithelial cells by HEMA. Biomater. 2005; 26: 745-753.
- 56. Samuelsen JT, Dahl JE, Karlsson, S, Morisbak E, Becher R. Apoptosis induced by the monomers HEMA and TEGDMA involves formation of ROS and differential activation of the MAP-kinases p38, JNK and ERK. Dent Mater. 2007; 23: 34-39.
- 57. Samuelsen JT, Holme JA, Becher R, Karlsson S, Morisbak E, Dahl JE. HEMA reduces cell proliferation and induces apoptosis in vitro. Dent Mater. 2008; 24: 134-140.

- 58. Paranajpe A, Cacalano NA, Hume WR, Jewett A. Mechanism of n-acetyl cysteine-mediated protection from 2-hydroxyethyl methacrylate-induced apoptosis. J Endodontics. 2008; 34(10): 1191-1197.
- 59. Gauthier MA, Simard P, Zhang Z, Zhu XX. Bile acids as constituents for dental composites: in vitro cytotoxicity of (meth)acrylate and other ester derivatives of bile acids. J Soc Interf. 2007; 4(17): 1145–1150.
- 60. Ergun G, Egilmez F, Cekic-Nagas I. The cytotoxicity of resin composites cured with three light curing units at different curing distances. Med Oral Patol Oral Cir Bucal. 2011; 16 (2): 252-259.
- 61. Beriat NC, Ertan AA, Canay S, Gurpinar S, Onur MA. Effect of different polymerization methods on the cytotoxicity of dental composites. Eur J Dent. 2010; 4: 287-292.
- 62. Amirouche Korichi A, Mouzali M, Watts D. Effects of monomer ratios and highly radiopaque fillers on degree of conversion and schrinkage-strain of dental resin composites. Dent Mater. 2009; 25: 1411-1418.
- 63. Luiz BKM, Amboni RDMC, Prates LHM, Bertolin JR, Pires ATN. Influence of drinks on resin composite: Evaluation of degree of cure and color change parameters. Polym Test. 2007; 26: 438-44.
- 64. Viljanen EK, Skrifars M, Vallittu P.K. Dendric copolymers and particulate filler composites for dental applications: Degree of conversion and thermal properties. Dent Mater. 2007; 23: 1420-1427.
- 65. Franz A, König F, Lucas T, Watts DC, Schedle A. Cytotoxic effects of dental bonding substances as a function of degree of conversion. Dent. Mater. 2009; 25: 232-239.
- 66. da Silva EM, Poskus LT, Guimarães JGA, de Araujo Lima Barcellos A, Fellows CE. Influence of light polymerization modes on degree of conversion and crosslink density of dental composites. J Mater Sci: Mater Med. 2008; 19: 1027-1032.
- 67. Atai M, Motevasselian F. Temperature rise and degree of photopolymerization conversion of nanocomposites and conventional dental composites. Clin Oral Invest. 2009; 13: 309-316.
- 68. Howard B, Wilson ND, Newman SM, Pfeifer CS, Stansbury JW. Relationship between conversion, temperature and optical properties during composite photopolymerization. Acta Biomater. 2010; 6: 2053-2059.
- 69. Ogunyinka A, Palin WM, Shortall AC, Marquis PM. Photoinitiation chemistry affects light transmission and degree of conversion of curing experimental dental resin composites. Dent Mater. 2007; 23: 807-813.
- 70. Leprince JG, Leveque P, Nystern B, Gallez B, Devaux J, Leloup G. New insight into the "depht of cure" of dimethacrylane-based dental composites. Dent Mater. 2012; 28: 512-520.

- 71. Cerveira G.P, Berthold T.B, Souto AA, Spohr AM, Marchioro EM. Degree of conversion and hardness of an orthodontic resin cured with light-emiting diode and a quartz-tungsten-halogen light. Eur J Orthod. 2010; 32: 83-86.
- 72. Jagdish N, Padmanabhan S, Chitharanjan AB, Revathi J, Palani G, Sambasivam M, Sheriff K, Saravanamurali K. Cytotoxity and degree of conversion of orthodontic adhesives. Angle Orthod. 2009; 79(6): 1133-1138.
- 73. Eliades T, Eliades G, Brantley WA, Johnston WM. Residual monomer leaching from chemically cured and visible light-cured orthodontic adhesives. Am J Orthod Dentofac Orthop. 1995; 108: 316-321.
- 74. Gioka Ch, Bourauel Ch, Hiskia A, Kletsas D, Eliades T, Eliades G. Light-cured or chemically cured orthodontic adhesive resin? A selection based on the degree of cure, monomer leaching and cytotoxicity. Am J Orthod Dentofac Orthop. 2005; 127(4): 413-419.
- 75. Pithon MM, dos Santos RL, Martins FO, Romanos MTV, de Souza Araujo MT. Evaluation of cytotoxicity and degree of conversion of orthodontic adhesives over different time periods. Material Res. 2010; 13(2): 165-169.

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The prevalence and related risk factors of undescended testis in preschooler ans school-age boys in Kayseri, Turkey

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Abstract

Aim: Undescended testis may lead to infertility and malignancy if left untreated in early childhood. The purpose of this study was to determine the prevalence of undiagnosed undescended testis and related risk factors in preschooler and school-age boys in Kayseri Turkey.

Material and methods: This cross-sectional study was performed with totally 4032 male students aged between 5-16 years from 24 primary schools in Kayseri. The diagnosis was made generally by physical examination. Diagnoses were corrected with ultrasonography if necessary. Khisquare and Fisher Exact Test was used for statistical analysis. A p value <0. 05 was accepted as significant.

Results: The prevalence of undescended testis was determined as 2.2%. The rate of children with circumcision was 88.0%. Undescended testis was determined significantly high among uncircumcised boys; families from lower socioeconomic status, age between 5-6 years old and children with unliterated father. Age and father's education level were also detected as related factors with undescended testis in a model of logistic regression analysis.

Conclusion: It draws attention that there are still cases with undiagnosed undescended testis in preschooler and school-age boys around our city. Health screening programs planned on early childhood, especially for children with low father education level, may be more beneficial in the diagnosis and treatment of undescended testis in the region of Kayseri.

Key words: Undescendend testis, prevalence, risk factors, boy.

Introduction

Undescended testis is a disease that can easily be detected with a simple examination during follow-up of children's development1. Severe complications such as infertility, malignancies and psychological issues especially in adolescents may occur in undiagnosed and untreated cases. Early diagnosis is possible with screening programs which is a simple physical examination ¹⁻⁵.

Undescended testis, which is the most important cause of male infertility and genital cancer, is maldescent of one or both testicles into the scrotum. Undescended testis is observed in 1-5% of male newborns ⁶. Bilateral rather than unilateral undescended testis had an increased risk for development of malignancy. Undescended testis left in the abdomen or inguinal canal, has 74% risk of developing seminoma. Retractile testis, most frequent condition in the differential diagnosis of undescended testis, is high scrotal placement of testis due to increased reflex activity of cremasteric reflex. A testis which can readily move or be moved between the scrotum and canal in first months of life is referred to as retractile. The percentage of development of infertility in patients with retractile testis, unlike undescended testis, is same as normal population ^{7,8}.

The frequency of circumcision in school-aged children is around 80% in Turkey ¹²⁻¹⁴. Early diagnosis of undescended testis is a useful strategy during circumcion especially if applied in early ages with simple urogenital physical examination. Some genetic and environmental risk factors such as prematurity, parent's education leveland paternal age has been reported⁶.

There are examples of regional research on the prevalence of undescended testis in Turkey ^{1,3,4,5,12}.

However, there are no community-based epidemiological studies evaluating the prevalence of undescended testis and the risk factors in schoolchildren. The aim of this study was to determine the prevalence of undiagnosed undescended testis and related risk factors in preschooler and school-age boys in Kayseri, Turkey.

Material and methods

This cross-sectional study was carried out on 4032 boys in between March 2007 and June 2007 at 24 primary schools located in the center and districts of the province of Kayseri, Turkey. According to the records of the Provincial Directorate of National Education in 2007, the number of primary school was 241; the number of male students studying in these schools was 71720. Primary schools were grouped, as good, moderate and poor according to the socioeconomic and development zones they represented. Due to population characteristics of Kayseri, 24 primary schools were randomly selected from the Provincial Directorate of National Education lists sampling 10% of the population representing 30% of rural and 70% of urban population. Approval from the research committee and from the parents was obtained.

Children were examined with the same physician. One hundred ninety four children with suspected undescended testis with clinical examination were confirmed with ultrasonography by an expert radiologist.

The statistical analysis was conducted using the SPSS 15.0 package program (Chicago, IL). Data were defined as number and percentage. Categorical variables were analyzed using the chi-square test and Fischer Exact Test. Logistic regression analysis with Backward LR method was used for detecting variables with undescended test. A p value less than 0.05 was accepted as statistically significant.

Results

The prevalance of undescended testis was 2.2%. The sociodemographic characteristics of children in the study group were given in Table 1. There was a statistically significant difference between age groups (p < 0.001), socioeconomic

levels (p <0.001), fathers' education levels (p = 0.002), and circumcision (p = 0.038) in terms of frequency of undescended testis (Table 2). There was no statistically significant difference in terms of other variables (p>0.05).

Binary comparisons of age groups revealed that there was no statistically significant differen-

Table 1.	Sociodemographic	features	of	children
(n=4032))			

Footures	Number	Doroont (0/)
A ga (year old)	Tuniber	Tercent (70)
Age (year old)	138	3.1
7 12	2846	70.6
12 16	2040	70,0
13-10 Family Structure	1040	20.0
Nuclear Family	2042	75 /
	<u> </u>	73.4
Large Family	990	24.0
Mother Occupation	2015	07.1
Employed	3915	97.1
Unemployed	11/	2.9
Father's Occupation	1015	25.2
Employed	1017	25.2
Unemployed	3015	74.8
Socioeconomic Status		
Poor	1018	25.2
Moderate	2719	67.4
Good	295	7.3
School Settlement		
Urban	1281	31.8
Rural	2751	68.2
Number of Siblings		
1-2	1334	33.1
3-4	2055	51.0
5 and above	643	15.9
Father's Education Level		
Unliterated	806	20.0
Primary School	2614	64.8
High School and above	612	15.2
Mother's Education Level		
Unliterated	798	19.8
Primary School	2631	65.3
High School and above	603	15.0
Circumcision		
Yes	3547	88
No	485	12

			Undesc	ended		
Relat	ted Factors	Total	Tes	tis	p*	p*
			n	%		
	(5-6) (I)	138	6	4.3		I-II=0.282*
Age (year)	(7-12) (II)	2846	78	2.7	< 0.001	I-III=0.001 ^{&}
Age (year) Socioeconomic Status	(13-16) (III)	1048	5	0.5		II-III=<0.001
	Poor (I)	2066	72	3.5		I-II=<0.001
Socioeconomic Status	Moderate (II)	1736	12	0.7	< 0.001	I-III=0.295
	Good (III)	230	5	2.1		II-III=0.040 ^{&}
	Unliterated (I)	174	12	6.9		I-II=0.001
	Literated (II)	524	10	1.9		I-III=0.001*
Fother's	Primary School (III)	1936	40	2.1		I IV = 0.001
rather s	Secondary School (IV)	678	13	1.9	0.002	1-1 V - 0.001
Education Level	High School (V)	438	9	2.1		I-V=0.003
	University (VI)	202	5	5 1.0		I-VI=0.005
		282	5	1.0		Other>0.05#
Circumaisian	Uncircumcised	485	17	3.5	0.029	
Circumension	Circumcised	3547	72	2.0	0.038	

Table 2. Related Factors Affecting the Prevalence of Undescended testis (n=4032)

* Chi-Square test; & Fisher's Exact test; # No significant difference was detected between subcategories of father's education levels.

Table 3.	Logistic	Regression	Analysis	of relate	ed factors	of	[•] Undescended	Testis
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Variable (Reference)		B [¥]	OR [€] (%95CI*)	Р
Age (13-16 year)				
	5-6	2.1	8.1 (2.4-27.3)	0.001α
	7-12	1.6	5.4 (2.1-13.4)	<0.001 °
Father's Education University				
	Unliterated (I)	1.6	5.0 (1.6-15.7)	0.005 α
	Literate (II)	-0.1	0.9 (0.3-2.7)	0.907
	Primary School (III)	0.2	1.1 (0.4-3)	0.748
	Secondary School (IV)	0.1	1.1 (0.3-3.1)	0.869
	High School (V)	0.1	1.1 (0.3-3.4)	0.820
Economy Good				
	Poor	0.6	1.9 (0.6-5.8)	0.239
	Moderate	0.9	2.5(0.9-6.8)	0.059

* CI: Confidence Interval. [#]B=regression coefficient. ^eOR=Odds Ratio, ^a p<0.05

ce between 5-6 age group and 7-12 age group. The difference between age groups was because of the low prevalence of undescended testis in 13-16 age group (p<0.05).

Although there was a statistically significant difference between poor and moderate socioeconomic status of the children (p<0.001); no statistically significant difference was detected between poor and good socioeconomic status of the children (p>0.05).

There was a statistically significant difference between groups in terms of fathers' education level and this difference was due to the high frequency of undescended testis in children with unliterated father (Table 2).

The model obtained from logistic regression analysis for determining the factors affecting the prevalence of undescended testis revealed that age, parental education and economic status were related factors for undescended testis (Table 3).

Discussion

In this study, the prevalence of undescended testis in preschooler and school-age boys were found to be 2.2%. The prevalence of undescended testis was higher in uncircumcised children, poor family socioeconomic status, 5-6 age group and children with unliterated father. Thus, the model obtained from logistic regression analysis showed that age and father's educational level were effective factors with the prevalence of undescended testis.

Undescended testis is one of the most encountered abnormalities at birth and the risk of developing malignancy increases if not detected and treated early in childhood ¹⁵⁻¹⁷. The prevalence of undescended testis in term newborns and children older than one year of age are 4-9% and 1-2% respectively⁹. Zivkovic et al. reported the prevalence of undescended testis as 2.0% in a screening of program of school children ¹⁸. Similarly, Schnack et al. reported the prevalence of undescended testis in children older than one year old as a 2.48% in a study conducted on approximately 1 million boys ¹⁹. In our country, Kayıkçı et al. reported the prevalence of undescended testis in Duzce, Turkey as 1.1%; Varol et al. in Samsun, Turkey as 1.2% which was similar ^{3,12}.

In our study, there was no difference in the prevalence of undescended testis among preschooler and elementary school children. There were 83 children with undescended testis in 7 year and older children. Of those, 5 children were in 13 year old and older age group. It was 8.1 fold (95% CI 2,4-27,3) higher in 5-6 age group than 13-16 age group. Similarly, Zivkovic et al. reported only two children with undescended children higher than 13 year old 18. Reduction in the incidence of undescended testis at advanced ages may be due to diagnosis and treatment in the early stages of childhood.

In our study, the frequency of undescended testis in children with unliterated father was five fold (95% CI 1.6 to 15.7) higher compared to fathers with a university degree. Sivasli et al. reported that low father's education level shifts the circumcision age of children to older age 20. The high frequency of undescended testis in uncircumcised children supports this finding. In the traditional Turkish family structure, the role of father in deciding circumcision is more effective. For this reason, circumcision may be unregarded from fathers of low educational level and may be delayed to contact a physician that may result in delayed detection of undescended testis. In addition, due to sociocultural reasons, families with older boys may wait for circumcision until minors grown up which may be another reason for delayed diagnosis.

We demonstrated that the presence of undiagnosed undescended testis in preschool and primary school age children may demonstrate that parents, health care workers and, educators still have not enough knowledge about undescended testis and there are insufficient health screening programs for children for this issue in this country.

In conclusion, parents, health professionals and educators have the responsibility for early detection of undescended testis in preschooler and school-age children. Informative, comprehensive local and national training programs for families, children, physicians, and educators throughout the country may play an important role in the early diagnosis and treatment of this disease. Early age health screening programs for fathers of low edcuational level may be more beneficial.

References

- Akay A.F, Şahin H, Em S, Kuru A.F, Ayçiçek S, Bircan M.K. [The Rates Of External Genital Organ Anomalies in Primary School Boys In Diyarbakir]. Türk Üroloji Dergisi 2002; 28 (1): 76-79.
- Köroğlu E, Karaaslan Y, Yöneyman F, Gürvit G, Yusuf M. Ro-CODEC Çocuklarda kronik hastalıkların sıklığı tarama çalışması. Roche® adına Yöneyman F, Gürvit G, Yusuf M, Köroğlu E, Karaaslan Y. Ankara, MedicoGrafics®, 1997.
- 3. Kayıkçı M.A, Çam K, Akman R.Y, Erol A. [The Ratio Of External Genital Anomalies In Male Children Attending Primary School in Duzce]. Türk Üroloji Dergisi 2005;31(1): 79-81.
- 4. Remzi D, Çakmak F, Erol D: [The Frequency of External Genital Organ Diseases and the Congenital Abnormalities in Healthy Adolescent Boys Living in Turkey]. Hacettepe Tip Cerrahi Bülteni 1980; 13(3):269-273.
- Altunoluk B, Bakan V, Özer A, Malkoc O, Gul A.B, Erkan E et al. [The prevalence of external genital organ anomalies among the male students attending primary schools in Kahramanmaras]. Türk Üroloji Dergisi - Turkish Journal of Urology 2010;36(1):87-90.

- 6. Pierik FH, Burdorf A, Deddens JA, Juttmann RE, Weber RF. Maternal and paternal risk factors for cryptorchidism and hypospadias: a case-control study in newborn boys. Environ Health Perspect. 2004;112(15):1570-1576.
- 7. Wood HM, Elder JS. Cryptorchidism and testicular cancer: separating fact from fiction. J Urol 2009; 181(2): 452-461.
- 8. Yılmaz Y, Ozen İ.O. [İnmemiş Testis Kliniğinde Güncel Yaklaşımlar]. STED 2004;13(6): 211-214.
- 9. Boisen KA, Kaleva M, Main KM, Virtanen HE, Haavisto AM, Schmidt IM. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. Lancet 2004;363:1264-9.
- 10. Zorludemir Ü. [Inguinoscrotal pathologies]. Türk Ped Arşivi 2010; 45 Özel Sayı: 23-28
- 11. Söylet Y. [Penis anomalileri]. Türk Ped Arşivi 2010; 45 Özel Sayı: 94-99
- 12. Güçer H, Bağcı P, Uzun H. [Testiküler Epidermoid Kist: Olgu Sunumu]. Fırat Tıp Dergisi 2010; 15, (3): 155-157
- Varol S, Özden E, Bostancı Y, Yakupoğlu YK, Yalman C, Sarıkaya Ş. [The testicles and penis sizes and rates of external genital organ anomalies in primary school boys in Samsun]. Türk Pediatri Arşivi, 2011; 46: 159-163.
- 14. Yurdakök M. [Information about pediatrics in Holy Books]. Kutsal kitaplarda çocuk hekimliği bilgileri. Ankara, 2001. Alp Ofset Makine San. ve Tic. Ltd. Şti: 2001: 65-92
- 15. Tekgül S. [Circumcision]. Çocuk Sağlığı ve Hastalıkları Dergisi 2000; 43: 297-302
- 16. Kaleva M, Toppari J. Cryptorchidism: an indicator of testicular dysgenesis? Cell Tissue Res. 2005 Oct;322(1):167-72.
- 17. Pike MC, Chilvers C, Peckham MJ. Effect of age at orchidopexy on risk of testicular cancer. Lancet 1986;1:1246-8.
- Pottern LM, Brown LM, Hoover RN, Javadpour N, O'Connell KJ, Stutzman RE et al. Testicular cancer risk among young men: role of cryptorchidism and inguinal hernia. J Natl Cancer Inst 1985;74:377-81.
- Zivkovic D, Varga J, Grebeldinger S, Dobanovacki D, Borisev V. External genital abnormalities in male schoolchildren: an epidemiological study. Med Pregl. 2004;57(5-6):275-278.

- Schnack TH, Zdravkovic S, Myrup C, Westergaard T, Wohlfahrt J, Melbye M. Familial aggregation of cryptorchidism--a nationwide cohort study. Am J Epidemiol. 2008 Jun 15;167(12):1453-7.
- 21. Sivaslı E, Bozkurt A.İ, Ceylan H, Coşkun Y. [Knowledge, attitude and behavior of parents regarding circumcision in Gaziantep]. Çocuk Sağlığı ve Hastalıklar Dergisi 2003; 46: 114-118.

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The study on community health services emands of empty nest elderly in China

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Abstract

The number of empty-nest families is on the rise in China, especially in some town. Empty nest elderly is a special group in society, for unaccompanied and unattended, usually creates many problems in the physical, psychological, social. In this study, The 36-Item Short-Form Health Survey(SF-36), activities of daily living (ADL) and the community health services satisfaction scale were used to investigate the community health service demand and supply of 358 empty nesters. The results showed that the ability of self-care were worse and needed more nursing or take care of the others in the empty nest elderly; the empty nest elderly, in comparison with the not empty nest elderly, had lower physical and mental scores. With the age changing, the requirements of community health services were different in empty nest elderly, the satisfaction of community health service is low in the empty nest (39.1%). The utilization rates of community health services were low.

Key words: Empty nest elderly, community, health services.

Introduction

With the social and economic development, population aging has become an irreversible global trend in the twenty-first century (Wu et al. 2010). There are 60-year-old and older population of 134 million and will be estimated to increase to 740 million by 2020. With the age increasing, more and more old man became empty nesters (Liu, and Guo 2008). The empty nesters were lived alone and their children separated their parents. Empty nesters were the performance of social development and progress, and with the accelerating pace of population aging and the miniaturization trend of the family structure, the proportion of empty nesters will be to further improve (Xie et al. 2010). In China, the number of empty-nest families is on the rise, especially in some town. Empty nest are a special group in society, for unaccompanied and unattended, usually creates many problems in the physical, psychological, social (Liu, and Guo 2007). Community health service (Community Health, Services, CHS) is taking health as the center, taking the family as a unit and the community-wide, which is a the health service system of prevention, care, rehabilitation, health care, health education (Hou et al. 2012). The elderly, children and the disabled are the main service targets of community health service. With the aging of population, empty nest of rapid development, empty nester has gradually become an object group of community health service. The characteristics of empty nester were large quantity, more service project and difficulty, thus they will become work focus of the community health service. Compared with some developed countries, the development of chinese community health services is relatively late, the system is still not perfect (Zhou et al. 2011). In this study, the community health service demand and supply of empty nester were used to study to constantly improve the community health service. Which provide the scientific basis for the government and relevant departments, so as to establish a perfect community health services for the aged system, further improve the life quality of empty nesters.

Methods

Location

Jilin is located in the middle of Jilin Province, with a geographic area of 27,120 km2, a population of 433.3 million in 2011. Jilin has 4 districts (Chuanying District, Changyi, Longtan District, Fengman District), 18 community service centers.

Subjects

Multistage stratified random cluster sampling was used. First, 4 districts in Jilin were stratified

as two groups according to the developmental level of economy. Second, the community service center in every selected district were stratified as three groups according the developmental level of economy, and one community service center was randomly selected from every group. A standardized questionnaire was sent to the empty nesters.

A total of 801 empty nesters in Jilin County were invited to participate in the study, with informed consent after receiving information about the goal and the method of the investigation, together with approval from the local government authorities, 728 empty nesters completed the questionnaire thoroughly. 45 elders were not able to complete the questionnaire because of physical or mental disabilities, and 28 elders returned incomplete data. The response rate of effective questionnaires was 90.88% (728/801). In the study, elders were classified as empty nest group and non-empty-nest group by distinguishing, "Are you living with any of your children together?" If the answer was "no" instead of "yes", this was seen as an empty nester (Wang, and Zhao 2012).

Instruments

The 36-Item Short-Form Health Survey (SF-36), developed in the US and employed in population surveys around the world, has been shown to be a valid measure of general health in population studies. It provides scores for eight domains: physical function, role limitations—physical, vitality, bodily pain, role limitations-emotional, social functioning, mental health and general health perceptions. Domains are scored on a 0–100 scale with higher scores indicating better health. In addition, two summary scores are calculated from the subscale scores: the Mental Component Summary (MCS) and the Physical Component Summary (PCS)(Wu et al. 2010).

Activities of daily living (ADL) is composed with independent living table (physiscal self-maintenance scale, PSMS) and instrumental activities of daily liing scale (instrumental activities of daily living scale, IADL), it is used to evaluate the ability of daily life (Liu, and Guo 2007).

The community health services satisfaction scale was developed to assess the satisfaction of the residents on community service, based on the feature of elderly and community health services in Jilin, some appropriate contents were added (Xu et al. 2010). The contents of the questionnaires: (1) The status quo of community Health Services supply, including the existing health resource utilization, and community health services project. (2) The demands for community health services, including services provided by the community health service centers for the elderly. Questionnaire reviewed by the relevant experts, the reliability analysis was used Cronbach's and the coefficient was 0.85.

Data analysis

The association between SF-36 scores in both the empty nest and not empty nest elderly were explored with Pearson's correlations. We used the linear regression model with conditional stepwise analysis to study the significant factors predictive of loneliness. All data were analyzed with the SPSS 13.0 statistical analysis software package (Wong et al. 2010).

Results

General information

The resulting data included 358(49.2%) empty nest elder adults and 370 (50.8%) not empty nest elder adults. Ages of these respondents ranged from 60 to 81 years (mean = 61.7 ± 6.78 years) for the empty-nest group and from 60 to 83 years (mean = 63.1 ± 7.21 years) for the non-empty-nest group. Most of the empty nest and not empty nest elderly had finished their education to the primary school level, and had been in the medical system. There were no significant differences between the empty nest and not empty nest group in gender, age, educational level, marital status and insurance coverage (P>0.05)(Table 1). The empty nest elderly, in comparison with the not empty nest elderly, had lower physical (95%CI = .217 to 6.131, t = 2.208, df = 533) and mental (95%CI = .812to 6.281, t = 2.511, df = 523.7) scores from the SF-36(Table 1).

581 elders were suffered from chronic diseases (79.8%), 357 elders were suffered from one disease (49%), 114 elders had two kinds diseases (19.9%), 22 elders had three kinds diseases (15.7%) and 147 elders without evidence of disease (20.2%). The chronic diseases threatened the

Factors	Empty nest group (n=358, %)	Not empty nest group (n=370, %)	P value
Gender			
Male	48.3	45	>0.05
Female	51.7	55	
Age	61.7±6.78	63.1±7.21	
Educational level			
Post-secondary school	10.6	11.6	>0.05
Technical school	35.8	35.9	
Primary school	37.4	36.8	
No education	16.2	15.7	
Income			
Very low	10.1	10.4	>0.05
Low	11.2	11.9	
Middle	58.9	57.4	
High	10.7	10.2	
V ery high	9.1	10.1	
Married status			
Married	74.3	72.7	>0.05
Single	25.7	27.3	
Insurance			
Medicare	90.5	93.2	>0.05
Uninsured	9.5	6.8	
SF-36 scales			
Physical health subscale ^k	66.14 ±17.96	71.58 ± 17.87	>0.0.5
Mental health subscale ^d	6154 ± 17.84	72.45 ± 16.12	<0.05

Table 1. The socio-economic indicators between empty nest and not empty nest old subjects

a Score ranged from 0 to 100, with higher scores indicating better physical health

d Score ranged from 0 to 100, with higher scores indicating better mental health

Table 2. The comparison of community medical services between empty nest and not empty nest elderly

	Empty nest	group (n=358)	Not Empty nes		
	n	%	n	%	Р
Health examination	248	69.2	256	69.2	>0.05
Door medical services	212	59.1	170	48.1	<0.05
Health guide	218	60.9	221	59.7	>0.05
Domestic sickbed	96	26.8	57	15.4	<0.05
Rehabilitation care	68	19	71	19.1	>0.05
Other need	21	5.8	24	6.4	>0.05
No need	35	9.8	36	9.7	>0.05

Table 3. The comparison of community medicalservices between different age empty nest elderly

	60 years old group (n=138, %)	70 years old group (n=121, %)	80 years old group (n=99, %)
Health examination	33 (23.9)	21(17.4)	10(10.1)
Health guide	58(42)*	20(16.5)	9(9.1)
Door medical services	11(8)	11(9.1)	49(49.5)**
Domestic sickbed	8(5.8)	10(8.3)	19(19.2)
Rehabilitation care	20(14.4)	51(42.1) [#]	10(10.1)
Other need	4(2.9)	5(4.1)	2(2)
No need	4(2.9)	3(2.5)	1(1)

* denotes a significant difference to other services (P < 0.05)

denotes a significant difference to other services (P < 0.05)

** denotes a significant difference to other services (P < 0.05)

elderly health followed by hypertension (47.3%), diabetes (24.8%), osteoarthritis (10.8%), stroke (9.1%) and heart disease (8%).

The ability of daily life were decreased

Activities of daily living (ADL) is composed with independent living skills (physical self-maintenance scale, PSMS, including food, clothing, wash, and leaving the toilet, walk and a bath) and instrumental daily living skills (IADL, including phone, shopping, cooking, housework, laundry, use the traffic tools, medication and provide for oneself economy). The above 14 items daily life events were used in this study. "Do not help, some difficulties to need help and completely cannot provide for oneself" were used to evaluated the capability. The results showed that the ADL of elders drop as the growth of the age, the ability of self-care were worse. In comparison with the not empty nest, the empty nest elderly needed more nursing or take care of the others.

The comparison of community health service demands in different age empty nest elderly. The community health service demands were studied in different age empty nest elderly. The results showed that the 80 years old group urgent needed door medical services (49.5%), compared with other services, the differences were statistically significant (P<0.05); compared with other services, the demand of rehabilitation medical care was highest in 70 years old group (42.1%); the demand of health guid was highest in 60 years old group (42%); With the age changing, the requirements of community health services were different. The status of community health service supply Community health service center provided the diagnosis and treatment of disease, health care, prevention and rehabilitation treatment, health education and health services and other service. Only 98 empty nest elderly had medical services (27.3%), 71 empty nest elderly had health care services (19.8%), 68 empty nest elderly (18.9%) had prevention service, 34 empty nest elderly (9.5%) had rehabilitation services, 59 empty nest elderly (16.5%) had health education and health advisory service. The community health service center gave 56 empty nest elderly (15.6%) to establish health records health files. Only 69 empty nest elderly (19.27%) participated in the health education, health consulting, healthy knowledge exhibition which community health service center provided. At the same time, the satisfaction of community health service was studied, the results showed that 140 empty nest elderly were satisfactory with the community health services (39.1%), 218 empty nest elderly were not satisfactory with the community health services (60.9%). The results showed that the utilization rate of community health service was not high in the center.

Discussion

As the population aging in our country, the empty nest elderly has gradually become the object in the community health services, it would become the key work of community health service object (Hu et al. 2010). Some research found that the empty nest elderly had chronic disease rates as high as 73.0% and 43.3% empty nests cannot provide the life for oneself when they became 80 years old.

The sick and the life cannot provide for oneself made the empty nest old man need more help. Diagnosis and treatment of domestic sickbed demands were high in empty nest old man. This phenomenon hints that the community health service development space be very large (Yang et al. 2008). Compared with the not empty nest elderly, the empty nest elderly had poor psychological condition. Therefore, the community health service should pay attention to the empty nest elderly health services.

The results showed that the demands of the community health service have difference among different age empty nest elderly. 80 years old group were urgent needed door medical services (49.5%). This might be relative to two reasons. On the one hand, as the growth of the age, the rates of chronic diseases increased; on the other hand, the children of empty nest elderly were not at nearby, they were lack of family chaperone in sicken time, so the demand of door medical services is more urgent. 70 years old group and 60 years old group needed the demands of rehabilitation medical care and health guide, it might be relatively to higher culture, a certain economic income, small economic pressure in young empty nest elderly, they could take the initiative for health knowledge and have strong health concept and self-health care. Therefore, community health service center should actively explore new mode and a new method to adapt the various demands of empty nest elderly in different age levels (Su et al. 2012).

According to the results of this survey, in the community health service center provided service projects, the demands of empty nest elderly were as follows: regular health examination (69.2%), the door medical services (59.1%), health guide (60.9%) and rehabilitation health care (19%). only small amount old man take part in the regular health education and health services, some community had not begin domestic sickbed medical care and rehabilitation services. The supply of community health service, especially active supply had bigger difference. The main reasons are that China's community health service network is not still complete, inadequate investment, the function is not strong, personnel quality is not high, the impact is not big, those reasons all hindered the development of community health services (Wu et al. 2010).

The findings from our study could provide the government with some information about the demands of community health services in the empty nest elderly. Results of the study suggest that we need to be aware of the empty nest elder adults' demands in community health services (Wong et al. 2010). According to the actual situation in China, the publicity of community health service should be strengthen, through the billboards, regularly health seminars, the prevention and treatment of common diseases to raise the elderly self-care consciousness, self-health care and psychological health level (Hou et al. 2012). The health records were established for each empty nest elderly to take countermeasures of prevention and control measures to improve the quality of life among the empty nest elderly, some actions must be taken in the care for the elderly, especially for the empty nest elderly (Zhou et al. 2011).

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References

- 1. Hou, W., H. Fan, J. Xu, F. Wang, Y. Chai, H. Xu, Y. Li, L. Liu, B. Wang, J. Jin, and Z. Lu. (2012). Service functions of private community health stations in China: A comparison analysis with government-sponsored community health stations. J Huazhong Univ Sci Technolog Med Sci, 32(2): 159-66.
- Hu, H., W. Liang, M. Liu, L. Li, Z. Li, T. Li, J. Wang, T. Shi, S. Han, M. Su, X. Peng, Y. Peng, W. Zhao, B. Wang, P. Zhang, and W. Zhu. (2010). Establishment and evaluation of a model of a community health service in an underdeveloped area of China. Public Health, 124(4): 206-17.
- 3. Liu, L. J. and Q. Guo. (2007). Loneliness and healthrelated quality of life for the empty nest elderly in the rural area of a mountainous county in China. Qual Life Res, 16(8): 1275-80.
- 4. Liu, L. J. and Q. Guo. (2008). Life satisfaction in a sample of empty-nest elderly: a survey in the rural area of a mountainous county in China. Qual Life Res, 17(6): 823-30.
- 5. Su, D., X. N. Wu, Y. X. Zhang, H. P. Li, W. L. Wang, J. P. Zhang, and L. S. Zhou. (2012). Depression and social support between China' rural and urban emptynest elderly. Arch Gerontol Geriatr.
- 6. Wang, J. and X. Zhao. (2012). Empty nest syndrome in China. Int J Soc Psychiatry, 58(1): 110.
- 7. Wong, S. T., D. Yin, O. Bhattacharyya, B. Wang, L. Liu, and B. Chen. (2010). Developing a performance measurement framework and indicators for community health service facilities in urban China. BMC Fam Pract, 11: 91.
- 8. Wu, Z. Q., L. Sun, Y. H. Sun, X. J. Zhang, F. B. Tao, and G. H. Cui. (2010). Correlation between loneliness and social relationship among empty nest elderly in Anhui rural area, China. Aging Ment Health, 14(1): 108-12.
- 9. Xie, L. Q., J. P. Zhang, F. Peng, and N. N. Jiao. (2010). Prevalence and related influencing factors of depressive symptoms for empty-nest elderly living in the rural area of YongZhou, China. Arch Gerontol Geriatr, 50(1): 24-9.
- Xu, J., N. Wu, S. Jin, F. Wang, Y. Wang, L. Liu, and Z. Lu. (2010). Analysis of inpatient bed allocation equity and utilization in the city community health service center of China. J Huazhong Univ Sci Technolog Med Sci, 30(2): 141-4.

- 11. Yang, J., A. Guo, Y. Wang, Y. Zhao, X. Yang, H. Li, R. Duckitt, and W. Liang. (2008). Human resource staffing and service functions of community health services organizations in China. Ann Fam Med, 6(5): 421-7.
- 12. Zhou, W., Y. Dong, X. Lin, W. Lu, X. Tian, L. Yang, and X. Zhang. (2011). Community health service capacity in China: a survey in three municipalities. J Eval Clin Pract.

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Attitudes about epilepsy among adolescents from Novi Sad (Serbia)

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Abstract

Aim: The objective of this study was to assess the attitudes of 502 adolescent students (ages 15-17; both genders) about epilepsy.

Methods: This study used a questionnaire designed by Bozkaya and associates concerning attitudes towards epilepsy.

Results: Most of the students consider epilepsy to be a natural, non-contagious disease that should be treated by doctors, while half believe that epilepsy is an inheritable disease. More than three quarters feel that people with epilepsy can achieve success in life. A fifth of the students associated epilepsy with mental retardation. The students believe that having epilepsy is not an obstacle to working in law, business, or education, and that it is possible for epileptics to train in sports. Most of the students believe that epileptic children can play and socialize normally, and stated that they would behave normally in the presence of an epileptic. Approximately two thirds believe that people with epilepsy should be protected and treated with care.

Conclusions: The results of this study show that the respondents do not have discriminatory attitudes towards people with epilepsy. Attitudes toward epilepsy uncovered in this study area appear to be satisfactory, but suggest that continuous education of the entire society is necessary to ensure that epileptics are not subjected to social or professional discrimination.

Key words: Poll, epilepsy, epilepsy awareness, adolescent children.

Introduction

In neurophysiological terms, epilepsy is sometimes defined as sudden, rapid, excessive and localized activity of grey brain matter (1). Today, epilepsy is defined as paroxysmal and transient disturbances in brain function that start suddenly, stop spontaneously, and have a tendency to repeat. Epilepsy is typified by occasional episodes of disturbed consciousness, behavior, emotional reactions, and/or motor and sensory functions. Epileptic seizures (derived from the Latin, sacire –to take a possession of) are a clinical manifestation of the excessive number of abnormal, hypersynchronous neuronal discharges associated with epilepsy (2).

Attitude is a fundamental term in sociology and social psychology: studies of human attitudes are used to develop a better understanding of social life. (3). Attitudes can be thought of as a set of opinions that lead to the formation of fixed standards, which allow people to adequately respond to a diverse range of social and life situations. According to Rot, attitudes represent a set of permanent tendencies to react in a certain (positive or negative) way to some object (4). In general, attitudes are characterized by their dispositional character, complexity, and influence on behavior; as well as by how the particular attitude was initially formed.

Attitudes can be further differentiated according to attitude direction (valence), complexity, extremeness, compatibility, consistency, intensity (power) and significance (5). Although social and cultural norms create a certain level of accordance with respect to attitudes, our individual perceptions and experiences contribute significantly to the creation of a diversity of attitudes towards the same object (6). Attitudes can also be formed based on non-behavioral information about an object: for example, information from books, newspaper articles or television, as well as listening about the experiences of parents and friends. In numerous papers, for example Regan and Fazio (6), attitudes which are the result of direct experience with an object often lead to more consistency between the attitude and behavioral response. Moreover, such attitudes are more persistent over time, and more resistant to change, than attitudes formed by indirect experience.

Attitudes begin to form at birth, and are based on different experiences that mutually support a specific response, resulting in a predisposition to react a certain way to similar situations. Therefore, attitudes can be thought of as the result of socialization, and are adopted by social learning. In addition, individual motivations and personality characteristics play a significant role in the process of forming and changing attitudes.

Three factors appear to have a particularly important influence on attitude development: motivation, individual characteristics, and the specific social environment surrounding the individual (5,6). Attitude formation can also be affected by social comparisons with other individuals, their attitudes, capabilities and reactions, which are often used as a basis for evaluating personal knowledge, attitudinal capabilities, emotions and other states. For example, according to L. Festinger's theory of social comparison, evaluation of personal knowledge, attitudes, capabilities, behaviors and experiences is necessary in order to judge their correctness and appropriation (7). Furthermore, lack of information from some "objective reality" leads to self-evaluation via comparison with other people, whose behavior then represents a form of "social reality". Social comparisons among people can result in empathy (5,6). According to the literature, putting oneself in the mental frame of reference of another person requires recognition and identification with the current feelings, thoughts and reactions of a person that suffers, as well as simultaneous understanding of those reactions in the context of their importance and significance for the future of that person. The main objective of the present study is to determine the attitudes

of younger secondary school students from Novi Sad, Serbia toward epilepsy; and correlate potential differences with educational curriculum (medical-vocational school vs. all others) and gender.

Material and methods

The present study included 502 secondary school students (ages 15-17) of both genders from the first and second grade of high school. The polling sample consisted of 37.3% boys and 62.7% girls. Study participants were enrolled in one of six high schools in Novi Sad, Serbia and Vrbas Country, Serbia: 2 medical schools, 2 economy schools, 1 electro-technical school and 1 gymnasium. Of the participants, 48.8% were enrolled in medical vocational schools, while 51.2% attended other secondary school programs. The study was conducted from May to June 2011, and was based on a cross-sectional design. Before beginning the study, written consents were obtained from all school principals. All polling was voluntary and anonymous. To assess the attitudes of the students about epilepsy, study participants were given a questionnaire developed by Bozkaya et al. (8), which the second part deals with different attitudes towards epilepsy. Data collection was performed using specially designed software. Data obtained using questionnaires and by objective measurements were analyzed using the statistics program SPSS, version 18.0. Various descriptive statistical methods were used for data analysis, and the statistical significance of response differences were assessed using the χ^2 and Mann-Whitney test. Results with p≤0.05 were considered to be statistically significant.

Results

Nine tenths of the students (93.1%) consider epilepsy to be a natural disease. Differences in attitudes toward epilepsy with respect to gender and school program were not statistically significant (p > 0.05).

Approximately every second respondent has the attitude that epilepsy is a curable disease (42.2%), while a nearly equal number consider epilepsy to be incurable (39.4%), and 18.4% of the respondents were undecided. Significantly more female students and students enrolled in medical high school reported that epilepsy is an incurable disease. (Table 1). Slightly more than half of the respondents (52.8%) believe that epilepsy is an inheritable disease, while one fifth (18.2%) think that epilepsy is not inheritable, and 29.0% percent of the students had no opinion. No statistical differences with respect to school program or gender were found.

Approximately every second respondent (41.8%) believes that epilepsy is a lifelong disease. In particular, significantly more female students (χ 2=7.158, df=2, p=0.028) and students enrolled in medical high schools (χ 2=30.745, df=2, p<0.000001) responded that epilepsy is a lifelong disease (Table 2). Nine out of ten students responded that epilepsy is a contagious disease (90.5%). Differences in attitudes about epilepsy as a contagious disease with respect to gender and school program were not statistically significant (p > 0.05). Half of the respondents reported that an epileptic attack can happen without the patient losing consciousness. Differences in attitudes with respect to gender and school program were statistically significant: more female students (χ 2=9.489, df=3, p=0.023) and students enrolled in medical school (χ 2=29.975, df=3, p<0.000001) believe that a patient can retain consciousness during an epileptic attack (Table 3).

Eight out of ten respondents (83.2%) believe that people suffering from epilepsy can be as successful in life as other people. Differences in attitudes with respect to gender and school program were not statistically significant (p > 0.05). One fifth of respondents (21.9%) associated epilepsy with mental retardation, one sixth with insanity, and one sixth with an unsuccessful life (Figure 1). Differences in attitudes with respect to gender and school program were not statistically significant (p > 0.05).

Table 1. Student attitudes about epilepsy as a curable disease versus gender and school program

Enilongy is a	Gei	nder	School program		
curable disease	Male	Female	Medical high school	Other high schools	
Yes	54.4%	35.1%	30.9%	53.6%	
No	26.2%	47.4%	57.0%	21.8%	
Maybe	19.4%	17.5%	12.1%	24.6%	
Total	100.0%	100.0%	100.0%	100.0%	
р	0.001		0.000		

Table 2.	Student	attitudes	about	epilepsy	as a li	felong	disease vs.	gender	and school	program
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Enilonsy is a lifelong	Ger	ıder	School program		
disease	Male	Female	Medical high school	Other high schools	
Yes	34.6	45.6	57.1	26.1	
No	50.0	33.3	25.5	52.1	
Maybe	15.4	21.1	17.4	21.8	
Total	100.0	100.0	100.0	100.0	
р	p=0.028		p<0.000001		

Table 3. Responses	to the question	of whether patients	can remain conscious	during an epileptic attack
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Patients can remain	Gender		School program		
conscious during an	Mala	Esses	Madical high school	Other high	
epileptic attack	Iviale	remaie	Medical nigh school	schools	
Yes	29.3	45.9	55.6	25.0	
No	26.8	23.5	19.5	27.9	
Maybe	43.9	30.6	24.9	47.1	
Total	100.0	100.0	100.0	100.0	
р	p = 0.023		p<0.000001		

The student responders appear to believe that epilepsy does not affect working in the legal or education profession; while only a small percentage thinks that a person with epilepsy can become a pilot, soccer player or police officer (Figure 2). Differences in attitudes about professions available to people with epilepsy with respect to gender and school program were not statistically significant (p > 0.05). In contrast to many of the above responses, more male students ($\chi 2=6.501$, df=2, p=0.039), and students enrolled in non-medical high schools ($\chi 2=12.524$, df=2, p=0.002) believe that patients with epilepsy can become medical doctors (Table 4). Similarly, more female students ($\chi 2=10.244$, df=2, p=0.006), and students enrolled in medical high schools ($\chi 2=9.317$,

Table 4. Attitudes of the respondents about the possibility of people with epilepsy becoming medical doctors vs. gender and school program

Epilepsy patients can	Gender		School program		
become medical doctors	Male	Female	Medical high school	Other high schools	
Yes	64.5	50.8	52.1	59.0	
No	20.6	36.9	41.6	23.1	
Maybe	14.9	12.3	6.3	17.9	
Total	100.0	100.0	100.0	100.0	
р	p=0.039		p=0.002		

Table 5. Attitude toward the possibility of an epileptic becoming a police officer vs. gender and school program

Epilepsy patients can be	Gender		School program		
police officers	Male	Female	Medical high school	Other high schools	
Yes	45.9	26.4	25.8	40.6	
No	40.0	61.6	64.5	43.8	
Maybe	14.1	12.0	9.7	15.6	
Total	100.0	100.0	100.0	100.0	
р	p=0.006		p=0.009		

Table 6. Attitudes about people with epilepsy driving motor vehicles vs. gender and school program

Pooplo with opilopsy can	Gender		School		
drive motor vehicles	Male	Female	Medical high school	Other high	
urive motor venicles	wiate		Wiedical mgn senoor	schools	
Yes	28.1	50.0	59.0	28.4	
No	53.9	29.7	21.0	53.2	
Maybe	18.0	20.3	20.0	18.4	
Total	100.0	100.0	100.0	100.0	
р	p < 0.001		p<0.000001		

Table 7. Response to the question of whether people with epilepsy should be treated with extra protection and care vs. gender and school program

People with epilepsy	Gender		School program	
should be treated with extra protection and care	Male	Female	Medical high school	Other high schools
Yes	49.9	73.7	83.5	52.4
No	50.1	26.3	16.5	47.6
Total	100.0	100.0	100.0	100.0
р	p=0.000		p=0.000	

df=2, p=0.009) have the attitude that epilepsy is a factor which would prevent a person from becoming a police officer (Table 5). More respondents (81.40%) enrolled in medical high schools (χ 2=9.264, df=2, p=0.01) have a positive attitude about people with epilepsy working in the legal profession, in relation to respondents enrolled in other schools (63.40%), while differences with respect to gender were not statistically significant. No statistically significant differences were uncovered with respect to attitudes concerning epilep-



Figure 1. Association of epilepsy with other disorders or life problems



Figure 2. Attitudes about professions available to people with epilepsy



Figure 3. Attitudes about people with epilepsy participating in sports

tics becoming teachers, pilots, judges, or soccer players (p > 0.05).

With respect to sports, the majority of students believe that it is possible for epilepsy patients to train in the sports listed in the questionnaire; although nearly half of the respondents think epilepsy patients cannot participate in swimming (Figure 3). Differences in attitudes about people with epilepsy participating in sports were not statistically significant vs. gender and school program (p > 0.05). Less than half of the respondents (41.3%) believe that a person affected by epilepsy can drive a car. Notably, significantly more female students (χ 2=14.518, df=2, p<0.001) and students enrolled in medical high school (χ 2=28.658, df=2, p<0.000001) had positive attitudes about epilepsy patients driving motor vehicles (Table 6).

Interestingly, 98.0% of the respondents enrolled in medical schools and 90.1 % students from the other schools believe that children with epilepsy can play and socialize normally with their peers: a positive attitude was observed more often among students enrolled in medical schools ($\chi 2=6.1278$, df=2, p=0.047). Differences with respect to gender were not statistically significant (p > 0.05). Nine out of ten respondents (90.1%) reported that it would not bother them if someone in their class had epilepsy. No statistically significant differences were found with respect to gender or school program (p > 0.05). One third of the students (32.8%) think that a person with epilepsy should always be accompanied by another person, while 44.80% think that this is not necessary. Differences with respect to gender and school were not statistically significant (p > 0.05).

Nearly all of the respondents (97.1%) believe that epilepsy should be treated by a doctor, while one-in-nine believes that a priest could be involved in the treatment: only 4.1% of the students responded that epilepsy treatment should involve a healer. Differences with respect to gender and school program were not statistically significant (p > 0.05). Approximately two thirds of the students (64.9%) believe that one should treat people with epilepsy with extra protection and care. This protective attitude was statistically significantly more common among students enrolled in medical schools (Mann-Whitney Z=-4.749, p=0.000) and among female students (Mann-Whitney Z=- 3.606, p=0.000) (Table 7). The last two questions in the questionnaire dealt with attitudes toward the discrimination of people with epilepsy. Based on these responses, 98.8% of the students do not appear to discriminate against people with epilepsy. No statistically significant differences were found with respect to gender and school program (p > 0.05)

Discussion

Epilepsy is one of the most common neurological problems, with a prevalence rate of between 2.8-19.5 per 1000 people in the general population: representing an estimated 1% of the entire global disease burden. Epilepsy most commonly first appears in either the very young or the elderly, and people with epilepsy are under significantly increased risk for developing psychological problems, ranging from depression and anxiety to serious psychoses. Unfortunately, socio-cultural attitudes still have a negative impact on the treatment of epilepsy patients, especially in developing countries, where this disease is still connected to superstition, discrimination and stigmatization. This problem is compounded by the fact that, geographically, approximately 80% of epilepsy sufferers live in developing countries. However, based on the present study, the attitudes of the students questioned in this survey appear to be satisfactory. Furthermore, we propose that the formation of these attitudes was influenced both by the educational program the students were enrolled in, and the particular social surroundings in which the students were raised (urban).

The majority of the students (93.1%) consider epilepsy to be a natural disease, and four out of ten (42.2%) believe that epilepsy can be cured. These results are only somewhat less than those given by youth in Cameroon, where 62.2% believes that epilepsy is a curable disease (9). Approximately half of the respondents (52.8%) think that epilepsy is an inheritable disease: somewhat more than among children of similar age from a related study in Tanzania (46%) (10). Over forty percent of the respondents believe that epilepsy is a lifelong disease.Most of the students do not think that epilepsy is contagious (90.5%), in agreement with a related study by Zielinska et al. (11), in which as many as 91% of the responders did not consider epilepsy to be a contagious disease . Interestingly, attitudes about the contagiousness of epilepsy are completely different in developing countries (Cameroon, Tanzania, Burkina Faso) (9,10,12), where more than half of the respondents believes epilepsy is a contagious disease, transferable by saliva and physical contact. According to Millogo et al, (12) these beliefs could be the main reason for the discriminatory attitude of Burkina Faso high school students toward people suffering from epilepsy.

In the present study, the students mostly believe that having epilepsy does not influence the ability of a person to work in the legal or education professions; whereas only a small percentage of the students believe that, in spite of having epilepsy, a person can become a pilot or police officer, in agreement with the results of Bozkaya et al (8). On the other hand, young Egyptians see epilepsy as an obstacle to both getting married and choosing a profession (13). In Cameroon, 72.7% of the young respondents believe that people suffering from epilepsy are unable to work in certain professions (9). In another study investigating the attitudes of high school and college students from Rome, Italy about social problems encountered by patients with epilepsy, 56% reported that having epilepsy is a limiting factor when choosing a profession, and that 55% believe that epilepsy patients should not enter into military service (14). In a related study, 26.2% of students in Kuwait reported that they would not hire someone with epilepsy (15).

With respect to sports, most of the students from the present study think that it is possible for epileptics to train in the sports listed in the questionnaire, in agreement with the results of Mecarelli et al. (14). Slightly less than half of the respondents (41.3%) believe that a person with epilepsy can drive a car, significantly more than their peers in Italy, where only 18% approve of people with epilepsy driving automobiles (14). In addition, 93.4% believes that children with epilepsy can play normally and socialize with their peers. According to findings from a related survey conducted in Poland, 92% of students also think that children with epilepsy can play with their peers (11); whereas in Kuwait, 12.5% of the student responders would not allow their children to play with children suffering from epilepsy (15). In the present study, nine tenths (90.1%) of the students reported that they would behave normally in front of a classmate who suffers from epilepsy.

Approximately one third of the student responders (32.8%) think that people with epilepsy should be accompanied by another person.

One fifth of the respondents associated epilepsy with mental retardation, one sixth with insanity, and one sixth with an unsuccessful life. According to a related survey done in Kuwait (15), 10.5% of the students associated epilepsy with insanity, and in Poland 12% associated epilepsy with mental disease (11). In Cameroon, 38% see epilepsy as a mental disturbance (9), while in Tanzania, over 90% of the surveyed students associate epilepsy with below average intelligence (10). In the present study, nearly all of the respondents (97.1%) think that treatment of people with epilepsy should be done by doctor, while 11.3% believe that priests could be included in the treatment process, and 4.1% think healers should be involved. In contrast, in Cameroon, 65% of young people think that doctors should be responsible for treating epilepsy, while 9% would trust a healer and 30% think that epilepsy can be cured with God's help (9). The majority of high school students in Burkina Faso would choose traditional medicine to treat epilepsy (12).

In the present study, approximately two thirds of the student responders (64.9%) think that one should treat people with epilepsy with extra protection and care. Although results from the present study suggest that almost all of the respondents (98.8%) do not discriminate against people with epilepsy, this is opposite from results of surveys conducted in developing countries, where epilepsy is still associated with a high degree of stigmatization. For example, in Kuwait, 8% of respondents think that people with epilepsy should not get married, while 12% think that they should not have children. In the same survey, 56% of respondents said that they would not get married to a person suffering from epilepsy (15). In a related survey about knowledge and attitudes toward epilepsy among high school students in Asuit, Egipt, Ghaydaa and Dalia (13) observed a high degree of stigmatization, which is a result of misunderstanding and negative attitudes toward this disease. Based on their results, 28.4% of the respondents believe that people with epilepsy should not get married, and as many as 92% would not marry someone suffering from epilepsy. Reno et al. (16) proposed that, in order to remove the stigma associated with epilepsy, it is necessary to implement a health-education campaign, in order to disseminate correct information about this disease and thus improve attitudes toward epileptic patients.

Conclusions

Attitudes towards epilepsy in the examined area are satisfactory. However, further education of students is needed in order to disseminate accurate information about this disease, and thus improve attitudes towards epileptic patients. Our results suggest the need for future research that would analyze whether (and how) knowledge of people with epilepsy, or a person with epilepsy in the family, affects the formation of attitudes toward epilepsy.

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References

- 1. Jackson JH. On the anatomical, physiological and pathological investigation of epilepsies. West Riding Lunatic Asylum Medical Reports 1873; 3: 315–339.
- 2. Campbell C, Deacon H. Unravelling the Contexts of Stigma: From Internalisation to Resistance to Change. J Community Appl Soc. 2006; 16: 411-7.
- 3. Ajzen I. Attitudes, personality and behavior. Chicago: Dorsey Press; 1988.
- 4. Rot N. Psihologija ličnosti, Zavod za udžbenike i nastavna sredstva, Beograd; 2002.
- 5. Corrigan PW, River LP et al. Three strategies for changing attributions about severe mental illness, Schizophrenia Bull. 2001; 27: 187-195.
- 6. Regan DT, Fazio RH. On the consistency between attitudes and behavior: Look to the method of attitude formation. J Exp Soc Psychol. 1977; 13: 38–45.

- 7. Festinger L. A theory of social comparison processes. Hum Relat. 1954; 7: 117–140.
- 8. Bozkaya IO, Arhan E et al. Knowledge of, perception of, and attitudes toward epilepsy of schoolchildren in Ankara and the effect of an educational program. Epilepsy Behav. 2010; 17: 56–63.
- 9. Njamnishi A, Angwafor S et al. Secondary school students' knowledge, attitude and practice toward epilepsy in the Batibo Health District-Cameroon. Epilepsia. 2008; 50: 1262-5.
- 10. Matuja WB, Rwiza HT. Knowledge, attitude, and practice towards epilepsy in secondary school students in Tanzania, Cent Afr J Med. 1994; 40: 13-8.
- 11. Zielinska A, Klos E, Talarska D. Youth's knowledge and attitude to epilepsy. Rocz Akad Med Bialymst. 2005; 50: 99-101.
- 12. Millogo A, Kabore J et al. Sociocultural aspects of epilepsy, in secondary school students in Bobo-Dioulasso (Burkina Faso), Rev Neurol (Paris) 2002; 158: 1186-90.
- 13. Ghaydaa A Shehata, Dalia G. Mahran. Knowledge and attitude of epilepsy among secondary schools students (epileptic and non-epileptic) in Assiut city "Egypt", Epilepsy Res. 2011; 95: 130–5
- 14. Mecarelli O, Li Voti P et al. A questionnaire study on knowledge of and attitudes toward epilepsy in schoolchildren and university students in Rome, Italy. Seizure. 2007; 16: 313-9.
- 15. Al-Rashed H, Al-Yahya D et al. Knowledge of, perceptions of, and attitudes toward epilepsy among university students in Kuwait. Epilepsy Behav. 2009; 14: 367-71.
- 16. Reno BA, Fernandes PT et al. Stigma and attitudes on epilepsy a study: with secondary school students. Arq Neuro-Psiquiat. 2007; 65: 49-54.

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Histological analysis of rabbit sciatic nerve after intraneural application of lidocaine

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Abstract

Seven days after 4ml of 2% lidocaine were applied intraneurally, bilaterally into the sciatic nerves of 5 rabbits with the administration speed of 3ml per minute and the mean application pressure of 37.34 ± 25.12 kPa, we performed an intravital excision of the nerve segment from the application site and prepared it for histological analysis. Qualitative histological analysis showed changes in the connective tissue coverings of the nerves and the nerve fibers. The epineurium showed increased cellularity composed of mononuclear cells, numerous dilated and hyperaemic capillaries, bundles of collagen fibers with altered tinctorial properties and a more compact arrangement in the perifascicular zone. The perineurium was delaminated and the endoneural fibrovascular trajectories were pronounced and included numerous fibroblasts. The damage of nerve fibers was most prominent in the subperineural zones of the fascicle in case of intrafascicular applications. A greater degree of damage was recorded at the level of myelinated nerve fibers of wider diameter whose axons were dislocated, hyperacidofilic, and showing an advanced level of axiolysis up to the level of complete disintegration and the appearance of an anhist mass in the fiber structure. The myelin sheath showed enhanced acidophily in some areas and it sporadically lost structural arrangement. Mediumsized and thin myelinated as well as unmyelinated nerve fibers were dearranged, they had altered tinctorial properties of the axoplasm but they had a relatively preserved structure. Schwann cells were numerous, voluminous and partly separated from the fibers. Strong reactive changes at the level of epineurium and slightly less at the level of perineurium and endoneurium recorded at the level of light microscopy were confirmed by the findings of the stereological analysis. This quantitative method showed a significantly higher volume density of the epifascicular epineurium and higher volume densities of the perineurium and endoneurium in the intraneural group compared to the control group.

Keywords: Local anesthetic, application pressure, intraneural application, histological analysis, rabbit.

Introduction

Peripheral nerve injection injuries that arise during local anesthesia may result in serious complications and permanent neurological deficit (1, 2, 3, 4, 5). The degree of persistent neurological complication after regional anesthesia depends on the used technique, the most common being the injury of the brachial plexus, while the sciatic nerve injury was also observed. The degree of damage depends on the application site, the value of the application pressure, and the needle type as well as on the type and dose of the local anesthetic (2, 3, 4, 5, 6, 7, 8, 9, 10). In intraneural applications of local anesthetic, the level of damage is greater in intrafascicular than in the extrafascicular application (3, 4, 5). In the case of extrafascicular application changes are mostly localized in the epineurium in the sense of slight focal thickening of the epineurium at the application site with relatively preserved fascicle architectonics (11), and a late consequence may be endoneural and perineural fibrosis (7). Intrafascicular application of local anesthetic results in intense degeneration of myelinated nerve fibers of all the fascicle areas (11). Changes in nerve fibers after application of local anesthetic in the case of intrafascicular application are manifested in fragmentation and axonal swelling, and the intensity of damage increases with the application of higher doses of anesthetics and with the addition of epinephrine (12). Intraneural application of local anesthetic results in high values of the application pressure and delamelation and fragmentation of the myelin sheath and significant cellular infiltration (3, 4). Injuries of the nerve fibers are not only the result of direct action by the needle or direct neurotoxic effects of the local anesthetic, but they are also the result of indirect contribution of late endoneural and perineural fibrosis.

Material and methods

To perform this experiment, we used 10 male New Zealand White rabbits with an average weight of 2.8 kg. In 5 rabbits, we applied 2% lidocaine intraneurally, bilaterally into the sciatic nerve. Five untreated rabbits were used as control. Under general anesthesia and by preparing the operative field in aseptic conditions we surgically accessed the sciatic nerve in the gluteal region, right and left, 2-3 cm below the line that connects the greater trochanter and the tuberosity of the ischium. With a direct control by an optical instrument, we placed the 26G needle at an angle of 15-20° bilaterally into the nerve of 5 rabbits, and using an automatic infusion pump (PHD 2000, Harvard Apparatus, Holliston, MA) we applied 4 ml of 2% lidocaine intraneurally, bilaterally with the administration speed of 3ml per minute. Pressure data was analyzed using a software package (BioBench version 1.2, National Instruments, Austin, TX). Seven days after the injection, we performed an intravital nerve excision from the application site in the length of 1 cm.

The nerve samples were fixed in 10% buffered formalin and then prepared for the qualitative and quantitative histological analysis using hematoxylin-eosin (H&E) and azan staining. Qualitative histological analysis included all nerve areas and it was followed by stereological determination of the volume density of the nerve connective tissue coverings and nerve fibers by using an ocular with a built-in multipurpose measuring system M42 by Weibel and with a magnification of 250X and 1000X according to the instructions of Kališnik (13). Statistical analysis of the data obtained by the stereological method was performed using SPSS for Windows 13. Descriptive statistics was presented with the median and percentiles, minimum and maximum values, while comparisons between groups were made using the Mann-Whitney U-test. The values of p <0.05 were taken as statistically significant.

Results

During the intraneural application, spindleshaped nerve swelling, extending 1-2 cm proximally and distally from the application site, was observed at the application site in all nerves. Seven days after the application no visible signs of deep wound infection were noticed, and thickening of the epineurium was visible at the application site in 3 (30%) nerves (Figure 1). Mean values of the application pressure were 37.34 ± 25.12 kPa, they amounted to 18.70 ± 5.43 kPa extrafascicularly, and 55.97 ± 22.85 kPa intrafascicularly.

Qualitative histological analysis Control group

The epifascicular epineurium represents a narrow area of well-vascularized loose connective tissue surrounding the nerve and which continues in the loose and poorly structured cellular interfascicular connective tissue with occasional aggregations of adipocytes. The fascicles are re-



Figure 1. Thickening of the epineurium at the application site (arrow)
latively regular in shape, arranged singly or in groups and are wrapped in a fibrocellular perineurium which makes clear margins to the perifascicular connective tissue and to the closest nerve fibers. The nerve fibers have different diameters and have a relatively regular round form in crosssection. The myelinated nerve fibers of larger diameter dominate in the subperineural zones, while nerve fibers of medium and small diameter with a preserved myelin sheath and having uniform tinctorial properties of the axoplasm are predominant in the central areas. Intrafascicularly, the dominant



Figure 2. Control group. H&E, x 40



Figure 4. Intraneural (Intrafascicular) group H&E, *x* 40



Figure 6. Intraneural (Intrafascicular) group H&E, x 400

cellular populations are Schwann cells which are associated with the nerve fiber. Fibroblasts are rarely seen in the discrete endoneural areas (Figure 2, 3).

Intraneural group

In the area of direct application, the epineurium shows hypercelullarity composed mainly of mononuclear cells, with hyperaemic blood vessels, bundles of collagen fibers with altered tinctorial properties and a more compact arrangement in the perifascicular zone. The perineurium shows dela-



Figure 3. Control group. H&E, x 400



Figure 5. Intraneural (Intrafascicular) group. Azan, x 400



Figure 7. Intraneural (Intrafascicular) group H&E, x 100

mination, wherein it is significantly disintegrated at the application site, with the loss of demarcation to the surrounding perifascicular connective tissue and the closest nerve fibers, and the herniation of the fascicle structures in the perifascicular connective tissue. The cytoplasm of the perineural squamous cells is hyperacidofilic, and the nucleus is large and hyperchromatic. The blood vessels incorporated in the structure of the perineurium are dilated and hyperaemic. The fascicle nerve fibers in which the lidocaine was directly applied generally show damage, and the subperineural fibers show more extensive changes compared to the ones in the central areas. Myelinated nerve fibers of larger diameter are spatially rearranged, of increased voluminosity, their axons are dislocated, hyperacidofilic and showing an advanced level of axiolysis up to the level of complete disintegration and the appearance of an anhist mass in the fiber structure. Myelin sheath shows enhanced acidophily in some areas, and in places this envelope loses its structurality becoming a thin hyperacidofilic anhist ring. Medium myelinated, hypomyelinated

and amyelinated nerve fibers are dearranged, with altered tinctorial properties of the axoplasm but of relatively preserved structure. Schwann cells are numerous, voluminous and partly separated from the fiber structure. Endoneural fibrovascular trajectories are pronounced and include numerous fibroblasts (Figure 4, 5, 6 and 7).

In nerves in which lidocaine was applied extrafascicularly, the epineural connective tissue contains numerous fibroblasts and mononuclear cells between which are observed sporadically relicts of adipocytes and numerous dilated and hyperaemic capillaries in the application zone. Perineural lamellae are partly delaminated, the nuclei of their cells are hyperchromatic, and the collagen associated with the lamellae is extremely hyperacidofilic. Intrafascicular hypercellularity to which contribute voluminous Schwann cells and numerous fibroblasts can be observed. Myelinated nerve fibers of the subperineural zones are voluminous, their axons sporadically dislocated, showing an advanced level of axiolysis up to the level of complete disintegration and the appearance of an an-



Figure 8. Intraneural (Extrafascicular) group H&E, x 40.



Figure 10. Intraneural (Extrafascicular) group H&E, x 400.



Figure 9. Intraneural (Extrafascicular) group H&E, x 400



Figure 11. Intraneural (Extrafascicular) group H&E, x 400.

hist mass in the fiber structure. The myelin sheath has partly lost the structurality and it has become a thin anhist ring. Preserved myelinated nerve fibers of small diameter dominate in the central zones of the fascicle. Endoneural fibrovascular trajectories are pronounced (Figure 8, 9, 10 and 11).

Quantitative histological analysis

Statistical analysis (Table 1) of the average (median) volume density of the epifascicular epineurium showed a statistically significantly greater value in the intraneural group compared to the control group (U = 8.0, p = 0.001), while the average (median) volume density of the nerve fibers was statistically significantly lower in the intraneural group compared to the control group (U = 2.5, p <0.0005). Differences in the average (median) volume density of the interfascicular space between the two groups were not statistically significant (U = 39.0 p = 0.40). Differences in the average (median) volume density of the perineurium between the two groups were not statistically significant (U = 44.0, p = 0.64). Differences in the average (median) volume density of the endoneurium between the two groups were not statistically significant (U = 46.0, p = 0.76).

Discussion

Large fluctuations in the values of the application pressure are due to different application sites. If we place the needle into the loose structured epineurium, we get get lower values of the application pressure $(18.70 \pm 5.43 \text{ kPa})$ compared to the values obtained during intrafascicular application (55.97 \pm 22.85 kPa) because of a lesser mechanical resistance of the epifascicular connective tissue compared to the perineural lamellae. In Shanthaveerappa et al. (14) and Selander and Sjöstrand (15), this has also been seen as the reason of the recorded differences of the application pressures. Intrafascicular applications during which a rupture of the perineurium and leeking of the local anesthetic solution into the interfascicular space have occu-

Table 1. Volume densities of components (phases) of the sciatic nerve of the control group and intraneural group

		Volume density							
Analyzad					Percentiles				
component (phase)	Group	N	Min.	Max.	25th	50th (Median)	75th	Mann- Whitney U - test	р
Volume density of	Control group	10	0,07	0,27	0,09	0,13	0,17	8.0	0.001
epineurium	Intraneural group	10	0,11	0,45	0,27	0,30	0,35	8,0	0,001
Volume density of	Control group	10	0,18	0,48	0,26	0,31	0,34	30.0	0.40
space	Intraneural group	10	0,13	0,51	0,20	0,28	0,33	39,0	0,40
Volume density of	Control group	10	0,01	0,03	0,01	0,02	0,02	44.0	0.64
the perineurium	Intraneural group	10	0,02	0,06	0,01	0,03	0,03	44,0	0,04
Volume density of the endoneurium	Control group	10	0,08	0,10	0,07	0,07	0,09	46.0	0.76
	Intraneural group	10	0,06	0,12	0,06	0,08	0,10	40,0	0,70
Volume density of	Control group	10	0,33	0,51	0,40	0,43	0,48	2.5	<0.0005
the nerve fibers	Intraneural group	10	0,26	0,34	0,28	0,29	0,32	2,3	~0,0003

rred are considered as mixed i.e. intrafascicular and extrafascicular applications. During intrafascicular applications in which no rupture of the perineurium has occurred, we registered the highest values of the application pressure. Selander and Sjöstrand (15) applied 2% lidocaine mixed with the same amount of Evans blue solution to a definitive amount of 200µL with the speed of application 100-300µL/min, using a needle with the outer diameter of 0.3-0.4 mm into the rabbit sciatic nerve, and using the electric peristaltic infusion pump. They obtained both lower values of application pressure during extrafascicular application (3.3 to 7.9 kPa) and pressure values (39.9 to 99.7 kPa) during intrafascicular application. Somewhat higher values of application pressure that we recorded during extrafascicular application can be interpreted with the fact that we applied a higher volume and with a higher speed of application in our study, and we also placed the needle between a group of fascicles whose common perifascicular connective tissue, showing a more compact structure, provided greater mechanical resistance than the rest of interfascicular and epifascicular epineurium. Hadžić et al. (4) and Mornjaković et al (3) obtained an identical ratio of the pressure values, which was statistically significantly lower during extrafascicular compared to intrafascicular applications of lidocaine into the sciatic nerve of dogs, but these values were significantly higher for both treatment groups compared to the values that we obtained. In interpreting these differences it seems adequate to consider the specificity of structure and a significantly larger diameter of the sciatic nerve in dogs than in rabbits. In the genesis of peripheral nerve injury during intraneural application of local anesthetics Selander et al. (12) consider the direct trauma during application as the key determinant, while ischemia which leads to the disruption of the blood-nerve barrier and to the consequent appearance of the endoneural edema are crucial for Lundborg et al. (16). Pressure as a significant factor for nerve damage has also been quoted, considering that intraneural application results in different values of the application pressure (3, 4, 5, 6). Our data show that the degree of nerve structure damage after intraneural applications is directly proportional to the value of the application pressure. In the case of intraneural application we can observe macroscopically a spindle-shaped nerve swelling at the application site, which extends 1-2 cm proximally and distally. At the application site we also note a thickening of the epineurium. These findings are consistent with the descriptions of the nerve segment and its environment at the application site according to Hadžić et al. (4) and Selander et al. (12). After the extrafascicular application, the epineurium shows hypercelullarity composed of mononuclear cells, with plenty of fibroblasts, while blood vessels in these areas are dilated and hyperaemic. Myelinated nerve fibers from large fascicles were showing different degrees of damage of axons and myelin sheaths. Thereby, large myelinated nerve fibers were significantly damaged, especially in the subperineural zone, where they are dominantly present. The subperineural zone has a richer capillary network compared to the central zone of the same fascicle. Damage of the endothelial cells of blood vessels of the subperineural region, as more vulnerable components of the blood-nerve barrier, has apparently had a greater impact on changing the environment of nerve fibers in comparison to the central zone.

The endoneural fibrovascular trajectories are pronounced. Perineural lamellae were partly delaminated. Schwann cells generally are voluminous. Intrafascicular areas are hypercellular. Damage at the application site is more intense than at the opposite side. Our data are in accordance with the findings of Myers et al. (7), Lundborg (17), Partridge et al. (18), on the blood vessels and the perineurium, and the findings of Hadžić et al. (4) and Mornjaković et al. (3) regarding the intensity and distribution of myelinated nerve fiber damage after extrafascicular application of lidocaine into the dog sciatic nerve. Intrafascicular endoneural application leads to an increased endoneural pressure, appearance of edema and ischemia, resulting in a weak flow in the fascicle, hindered dilution and absorption, and longer longitudinal spread of the local anesthetic (12, 16, 19). Our qualitative histological analysis of the sciatic nerve in rabbits after intrafascicular application showed changes at the level of the nerve fibers, Schwann cells, blood vessels and intrafascicular connective tissue coverings of all treated nerves. The subperineural zones of the fascicle, into which was administered

the lidocaine, showed intensive damage in comparison to the central zones. Myelinated nerve fibers of larger diameter in the subperineural zone of the fascicle show a significantly higher degree of damage. Their myelin sheath is relatively often fragmented into small ovoid particles or their topographic location is anhist. Medium myelinated, hypomyelinated, and amyelinated nerve fibers, which are predominantly located in the central zone of the fascicle, show a smaller degree of damage. Myelinated nerve fibers on the opposite site of the application site were only partially degenerated. Endoneural fibrovascular trajectories are generally pronounced and they sequester plenty of myelin detritus at the the application site of the local anesthetic. Although our data is in accordance with several previously published studies that refer to the extent and distribution of nerve injury after application of a local anesthetic, there are also differences (3, 4, 8, 9, 11, 12, 19, 20, 21). We interpret these differences by the fact that previously no difference was made between intrafascicular and extrafacicular applications, for which we today know for sure that they result in different application pressures, and therefore different damage frequency. Stereological analysis of the sciatic nerve in the control group of rabbits was a precondition for the same kind of analysis of the sciatic nerve after intraneural application. Previous research at the level of light microscopy were primarily related to the myelinated nerve fibers (22, 23, 24, 25, 26, 27), and the unmyelinated nerve fibers were investigated using the electron microscopy (24).

Changes in the connective tissue structures of the nerve can result in late endoneural and epineural fibrosis, and indirectly cause damage of nerve fibers. Therefore, quantification of the degree of change in the connective tissue structures is significant for the decision making regarding the microsurgical repair of the nerve and thus to avoid subsequent damage of neural structures. Using stereological analysis, we found that the volume density of the epifascicular epineurium of the control group (0.13) was significantly lower (p = 0.001) than the volume density of the same component in the intraneural group (0.30). The volume density of the endoneurium of the intraneural group was higher than in the control group, but this difference was not statistically significant (p = 0.76). In comparison to the control group the volume density of the perineurium is larger in the intraneural group, but the difference was not statistically significant. Fascicle changes are significant only in the zone of the fascicle in which a local anesthetic was injected, and so exerting less influence on the volume composition of the treated nerve.

Change in the volume composition of the sciatic nerve after intraneural application of lidocaine in our animals indicates that they are predominantly a result of the proliferation of the nerve connective tissue coverings. These results of our quantitative histological analysis of the sciatic nerve of rabbits rely on the results of the qualitative histological analysis with which they are compatible. In conclusion, we can support the authors who consider the application site (3, 4, 7, 8, 9, 17, 28), the type and dose of local anesthetic (12, 18, 19, 29), and the value of the application pressure (3, 4) as the main factor in the genesis of nerve lesions during the procedure of local anesthesia.

Conclusions

During intraneural application, the values of application pressure are significantly higher in the case of intrafascicular in comparison to extrafascicular administration, wherein the degree of nerve damage is directly proportionate to the value of the application pressure.

During intraneural applications, more significant nerve lesions are detected in the case of intrafascicular in comparison to the extrafascicular application, and they are most prominent in the directly affected fascicles.

Thick-myelinated nerve fibers show a higher level of damage in comparison o medium myelinated, hypomyelinated, and amyelinated fibers.

The volume densities of the epifascicular epineurium during intraneural application are significantly higher in comparison to the values of the volume density of the untreated control nerves.

The volume density of the nerve fibers is statistically significantly higher in the control group in comparison to the intraneural group.

References

- 1. Cox B., Durieux M. E., Marcus M. A. Toxicity of local anesthetics. Best Prac Res Clin Anesthesiol. 2003; 17(1): 111-36.
- 2. Selander D. Neurotoxicity of local anesthetics: Animal Data. Reg Anesth. 1993;18(6): 461-8.
- 3. Mornjakovic Z., Dilberović F., Ćosović E. et al. Histological changes of the sciatic nerve in dogs after intraneural application of lidocaine-relation to the established application pressure. Bosnian Journal. 2005; 5(1): 8-13.
- 4. Hadžić A., Dilberović F., Shah S. et al. Combination of intraneural injection and high injection pressure leads to fascicular injury and neurologic deficits in dog. Reg Anesth Pain Med. 2004; 29(5): 417-423.
- Kapur E., Vuckovic I., Dilberovic F., Zaciragic A., Cosovic E., Divanovic K. A., Mornjakovic Z., Babic M., Borgeat A., Thys D. M., Hadzic A.Neurologic and histologic outcome after intraneural injections of lidocain in canine sciatic nerves. Acta Anesthesiol Scan. 2007; 51(1):101-7.
- 6. Ćosović E., Mornjaković Z., Šuško I., Aličelebić S. Quntification of some Qualitative Changes of the Rabbit Sciatic Nerve after the Application of lidocaine into the Mesoneurium. Med Arh. 2007; 61(4): 207-211.
- Myers R. R., Kalichman W. M., Reisner S. L., Powell C. H. Neurotoxicity of local anesthetics: altered perineurial permeability, edema and nerve fiber injury. Anesthesiology. 1986; 64: 29-35.
- 8. Gentili F., Hudson A., Kline G. D., Hunter D. Peripheral nerve injection injury: an experimental study. Neurosurgery. 1979; 4(3): 244-253.
- 9. Rice A. S. C., McMahon S. B. Peripheral nerve injury caused by injection needles used in regional anaesthesia: influence of bevel configuration, studied in a rat model. British Journal Anaesthesia. 1992; 69: 443-438.
- Nakamura T., Popitz-Bergez F., Biknes J., Strichartz G. R. The critical role of concentration for lidocaine block of peripheral nerve in vivo: studies of function and drug uptake in the rat. Anesthesiology. 2003; 99(5): 1189-97.
- 11. Gentili F., Hudson A., Kline D., Hunter D. Nerve injury with localanesthetic agents: A light and electron microscopic, fluorescent microscopic and horseradish peroxidase study. Neurosurgery. 1980; 68: 263-272.

- 12. Selander D., Brattsand R., Lundborg G., Nordborg C., Olsson Y. Local anesthetics: importance of mode of application, concentration and adrenaline for the appearance of nerve lesions. Acta Anesthesiol Scand. 1979; 23: 127-136.
- 13. Kališnik M. Temelji stereologije. Ljubljana: Društvo za stereologojo in kvantitativno analizo slike (DSKAS), 2002: 133.
- 14. Shanthaveerappa T. R., Bourne H. G. Perineural epithelium: a new concept of its role in the integrity of the peripheral nervous system. Science. 1966; 154: 1464-1467.
- 15. Selander D., SjÖstrand J. Longitudinal spread of intraneurally injected local anesthetics. Acta Anaesth Scand. 1978; 22: 622-634.
- 16. Lundborg G., Nordborg C., Rydevik B., Olsson Y. The Effect of ischemia on the permeability of the perineurium to protein tracers in rabbit tibial nerve. Acta Neurol Scandinav. 1973; 49: 287-291.
- 17. Lundborg G. Structure and function of the intraneural microvessels as related to trauma, edema formation, and nerve function. J Bone and Joint Surg. 1975; 57(7): 938-948.
- 18. Partridge B. L., Phil D. The effects of local anesthetics and epinephrine on rat sciatic nerve blood flow. Anesthesiology. 1991; 75: 243-251.
- 19. Kalichman W. M., Powell C. H., Myers R. R. Quantitative histologic analysis of local anesthetics-inducted injuri to rat sciatic nerve. J Pharmacol Exp Ther. 1989; 250(1): 406-413.
- 20. Mackinnon E. S., Hudson R. M., Bojanowski V., Maraghi E. Peripheral nerve injection injury with purified bovine collagen an experimental model in the rat. Annals of Plastic Surgery. 1985; 14(5): 428-436.
- 21. Dyck J. P. et al. Technique assessment of demyelination from endoneurial injection. Experimental Neurology. 1982; 77: 359-377.
- 22. Schwab B. W. et al. Rabbit sural nerve responses to chronic treatment with thalidomide and supidimide. Muscle Nerve. 1984; 7(5): 362-8.
- 23. Lopez I. et al. Estimation of the number of nerve fibers in the human vestibular endorgans using unbiased stereology and immunohistochemistry. J. Neurosci Methods.2005 Jun 30;145(1-2):37-46.
- 24. Larsen J. O. Stereology of nerve cross sections. J. Neurosci Methods. 1998; 85(1):107-18.

- 25. Gupta R., Rowshan K., Chao T., Mozaffer T., Steward O. Chronic nerve compressioninduces local demyelination and remyelination in rat model of carpal tunnel syndrome. Exp Neurol. 2004; 187(2): 500-8.
- 26. Hahn A. F., Chang Y., Webster H. D. Development of myelinated nerve fibers in the sixth cranial nerve of the rat: a quantitative electron microscope study. J Comp Neurol. 1987; 260(4): 491-500.
- 27. Schmitz C., Hof P. R. Desing-based stereology in neuroscience. Neuroscience. 2005; 130(4):813-31.
- 28. Myers R. R., Powell C. H., Costello L. M., Lampert W. P., Zweifach W. B. Endoneurial fluid pressure: direct measurement with micropipettes. Brain Research. 1978; 148: 510-515.
- 29. Zink W., Graf B. M. Toxicology of local anesthetics. Clinical, therapeutic and pathological mechanisms. Anaesthesist. 2003; 52(12): 1102-23.

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The impact of the body weight on aerobic capacity

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Abstract

Introduction: Overweight and obesity are defined as a health risk. One of submaximal tests which are suitable for beginners is Urho Kaleva Kekonen (UKK) test, the test we used to get the value of cardiovascular capacity (VO2max) of respondents.

Objective: The aim of this study was to determine the relationship between BMI and predicted values of VO_2 max estimated by UKK test for the first degree of obesity.

Methods: In this study, we first determine the BMI, and then perform the UKK test on treadmill. In this way, in laboratory conditions we obtained the value of the predicted VO2max.

Results: After a correlation analysis value obtained by UKK VO2max test and BMI for the third group provided the values of the correlation coefficient (r = -0.904, p < 0.001), which has also found an inverse dependence of these two values.

Conclusion: Low cardiovascular capacity and obesity can have a significant impact on the development of cardiovascular comorbidity.

Keywords: BMI, VO2max, UKK.

Introduction

Overweight and obesity are defined as increased or excessive accumulation of fat tissue which represents a health risk. Obesity is the one of the biggest problems in developed world. In a few of these cases, the cause of obesity is metabolic disorder, but in the most of cases the main problem is in energy disbalance. Simply put, energy consumption is smaller than the energy input. Obesity threatens mainly people older than 30 years, though, we also meet it among the young people. Medical History in obese persons should cover all data related to obesity itself in relation to the present comorbidity, diet and physical activities.¹

Generally, in obese persons, it is necessary to determine the degree of nutritional status or body mass index (BMI) or body weight index (BWI) which is expressed as a ratio of weight and height: BMI = BW / BH² (kg / m²)

Upon the recommendations of the World Health Organization, BMI is used for the classification of nutritional state by the following criteria:

- BMI < 18.5 undernourished person
- BMI = 18.5–24.9- normally nourished person
- BMI = 25 29.9 overweight
- BMI > 30 obesity
- BMI = 30–34.9 I degree (moderate) obesity
- BMI = 35 39.9- II degree (extreme, severe) obesity
- BMI > 40- III degree (extreme, morbid) obesity. ^{2,3,4}

An important step in the reduction of body mass is the exercise therapy. Walking is a type of aerobic exercise. Aerobic exercise is the type of exercise during which the essential parameter of VO2 max and cardiovascular abilities are observed. VO2 max is the maximum oxygen uptake or maximum volume of oxygen that is consumed in one minute during maximal exercise. It is presented in the milliliters of oxygen consumed in one minute per kilogram of body weight (ml / min / kg). It is often used as a parameter for the assessment of cardiovascular (aerobic) capacity. Physiologists have found that the determination of the amount of oxygen received, is the best measure for determination of the aerobic work. Such a determination requires expensive instruments and laboratory conditions. Tested person runs on a treadmill and volume of inhaled and exhaled air and heart rate are measured. To this end, important is the value of using a heart rate monitor (pulsmeter). By using it, we can easily and assurely supervise our aerobic training and achieve the desired results. Numerous tests are designed to measure or predict the maximum oxygen uptake (maximal aerobic capacity or VO2 max). Depending on the exhaustion of the subject during the test, the tests may be maximum or submaximum tests. ^{5,6,7,8,9}

One of submaximum test that is suitable for recreational sportists is Urho Kaleva Kekonen (UKK) test which provides the value of cardiovascular capacity (VO2max) in subjects. On basis of the obtained values of VO2max, one can calculate fitness index, and, based on the value of the fitness index, subjects are classified in a specific fitness categories based on which the appropriate physical activity are recommended. UKK test is suitable for the physically healthy persons (with no signs of infectious diseases, no chronic disease and with no osteomuscular damage or deformation) between 20 and 65 years of age. It is less reliable for persons older than 65 years, for the persons with severe and extreme obesity, and athletes often do not reach the required heart rate during the test. Because of that, this test is suitable only for those subjects with average activity (daily activities at home, regular jobs at the workplace and recreational activity twice a week). UKK original test was not designed as a typical test for the conveyor belt (treadmill). 10,11,12

The aim

The aim of this study was to determine the relationship between BMI and predicted values of VO2max obtained by UKK test for I degree of obesity.

Methods

Subjects

Three groups by ten male subjects of different ages and different physical status, voluntarily participated in the study. Criteria for the classification in a particular group was the value of BMI. Medical observation (ECG, heart rate, blood pressure, occupation, smoking habits, etc.) was performed before, during and 24 hours before testing. Subjects were instructed not to deal with difficult physical activities 48 hours before the test. The equipment was identical for all subjects. Environmental conditions (ambient temperature 21.3 ± 1.4 °C, humidity $42.4 \pm 7.5\%$) were the same for all subjects. The aim of this study and details of procedures applied in it, were explained and the opportunity to ask everything they didn't understand was given to all subjects. All procedures were performed in accordance with the ethical standards of the local Ethics Committee and the Helsinki Declaration. Method of research is UKK test, which provides the value of cardiovascular capacity (VO2max) in subjects after 2 km of fast walking, fitness index is calculated using a complex formula taking into account age, height, weight, test duration and heart rate to determine the physical condition of the subjects compared to the average values.

Fitness index = $420 + A \cdot 0.2 - (T \cdot 0.19338 + HR \cdot 0.56 + [W \div H^2 \cdot 2.6])$ for men,

Fitness index = $304+A \cdot 0.4-(T \cdot 0.1417+HR \cdot 0.32+[W \div H^2 \cdot 1.1])$ for women

A-age, H - height in meters, W-mass in kg, HR - the average value of heart rate during the test and T-time for 2 km of fast walking in seconds.

UKK obtained, provides the following parameters:

- 1. power consumption
- 2. fitness index
- 3. fitness category
- 4. predicted VO2max
- 5. predicted maximum heart rate

Based on the fitness index values, fitness categories are determined:

<70- significantly below average 70-90 - slightly below average 90-110 - average 110-130 - slightly above average > 130 - significantly above average

Pulse is recorded in 5-second intervals by Polar "heart rate" monitor (Polar 610i). Required equipment: treadmill, watch or stopwatch, heart rate monitor (POLAR 610i) and POLAR software, device for temperature and humidity measuring (KIMO HD200).

Description: subject walks for 2 km as fast as he can at a constant rate. On this basis, POLAR software automatically calculates the results of UKK test described above.

Statistical analysis:

The results are presented as arithmetic mean and standard deviation. Correlation analysis and determination of the correlation coefficient with reference to the table values for the appropriate level of probabilities of an appropriate level of degrees of freedom. The relation of BMI and VO-2max was evaluated using linear correlation test. The significance of differences between arithmetic means of the indicators was tested using the Student t-test.

Results

Table 1 shows the values of the first group of subjects who have a mean BMI 23.5 and the mean predicted VO2max 42.25 according the UKK test,

as a measure of cardiovascular (aerobic) capacity. There are also presented the values of a fitnesss index that are important to determine the fitness category. Table 2 shows the values of other group of subjects who have a mean BMI of 27.7 and the mean predicted VO2max 36.7 according to UKK test, as measures of cardiovascular (aerobic) capacity. There are also presented the values of a fitnesss index that are important to determine the fitness category. Table 3 shows the value of the third group of subjects who have a high value BMI 32.2 and the mean predicted VO2max 31.72 according to UKK test, as measures of cardiovascular (aerobic) capacity. There are also presented the values of a fitnesss index that are important to determine the fitness category. Since that this group of subjects represents a target category of

Table 1. The value of UKK test parameters and BMI of normal weight subjects

				Body	UKK		
Subject	Age	Weight	Height	Mass	Fitness	Predicted	Predicted
				index	index	HRmax	VO2max
1	22	70	191	19.2	94	198	47.4
2	29	78	192	21.2	101	191	47.5
3	41	75	185	21.9	89	188	38.9
4	22	81	192	21.9	81	199	42.4
5	23	67	171	22.9	104	184	51.5
6	27	83	187	23.7	95	190	46.3
7	39	89	189	24.9	81	187	36.2
8	39	93	193	24.9	87	190	38.2
9	30	91	191	24.94	84	187	37.3
10	35	95	195	25	83	187	36.8
Ā	30.7	82.2	188.6	23.05	89.9	190.1	42.25
SE	2.21	2.92	2.05	0.59	2.48	1.46	1.65

Table 2. The value of UKK test par	ameters and BMI of	overweight subjects
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				Body	UKK		
Subject	Age	Weight	Height	Mass index	Fitness index	Predicted HRmax	Predicted VO2max
1	43	85	180	26.2	79	186	39.8
2	34	86	180	26.5	84	185	39.0
3	32	88	182	26.6	95	185	44.7
4	30	90	183	26.9	84	186	40.8
5	38	90	180	27.8	76	185	34.9
6	36	96	185	28	62	190	30.1
7	34	101	190	28	90	182	41.4
8	39	98	186	28.3	70	183	31.6
9	40	100	185	29.2	75	183	33.3
10	38	96	180	29.6	67	184	31.4
x	36.4	93	183.1	27.7	78.2	184.9	36.7
SE	1.18	1.76	1.01	0.35	3.1	0.67	1.52

				Body	UKK		
Subject	Age	Weight	Height	Mass index	Fitness index	Predviđen HRmax	Predviđen VO2max
1	35	108	190	30.0	77	184	36.1
2	36	104	185	30.4	75	183	34.6
3	33	101	179	31,5	66	182	32.9
4	37	102	180	31.5	68	181	31.2
5	35	110	184	32.1	74	182	34.2
6	35	100	175	32.6	61	180	30.0
7	38	98	172	33.1	63	179	31.0
8	39	108	180	33.3	63	179	29.4
9	37	99	172	33.4	63	177	29.2
10	40	99	170	34.2	59	177	28.6
x	36.5	102.9	178.8	32.2	66.9	180.4	31.72
SE	0.64	1.3	1.97	0.41	1.9	0.72	0.78
r	- 0.904						
р	< 0.001						
DF	8						
t	- 5.98						

Table 3. The value of UKK test parameters and BMI of subjects with I degree of obesity

this research, the correlation analysis and Studenttest were performed with a corresponding probability and degree of freedom. After a correlation analysis of values of VO2max obtained by UKK test and BMI for the third group of subjects, the value of the correlation coefficient was provided (r = -0.904 p < 0.001), which means that there is a statistically significant correlation between VO-2max and BMI.

Discussion

On the basis of the obtaind parameters and on the basis of criteria of UKK test validity, subjects meet the range of 20-65 years of age and body weight ranged from 50-110 kg. All the subjects belong to part of the population that does not deal with active sport. The mean value of BMI (23.05 ± 1.88) for the first group of subjects, characterized them as the people with normal weight, and the average fitness index value of an (89.9 \pm 7.84) corresponds the value of fitness category 2 (slightly below average). The mean BMI value of the second group $(27.7 \pm 1:09)$ corresponds to the category of people with overweight and average fitness index (78.2 ± 9.79) belongs to the fitness category 2 (slightly below average). In the third group of subjects, mean BMI value is (32.2 ± 1.31) and this group belongs into the category of obese I degree (moderate) obesity, and they, according to the mean fitness index (66.9 ± 6.02), are in the fitness category 1 (significantly below average).

Comparing our results with those of Laxmi's studie in which he used Queen's College Step Test $(r = -0.48, p < 0.01)^{13,14,15}$, or results of the study in which Peter Pribis and colleagues used Astrand test (r = -0.334; p < 0.001)¹⁶, one can say that there is an inverse dependence (an increase in one variable, decreases the other variable) between the values of VO2max and BMI. In other words, increasing values of BMI result with decreasing values of VO2max. Common fact for the previously listed studies is that in every one of them a submaximum tests were applied. Poor aerobic capacity in young people who have a tendency for weight gain, can cause cardiovascular comorbidity in the middle age. ^{17,18,19} In last years, there were several studies about indirect indications of physical activity, such as walking during the day or any active participation in physical activities. ^{19,20,21,22} Physical activity causes certain positive effects on the entire body. It has an influence on body weight and body composition by causing weight loss and loss of body fat. It has effects on the cardiovascular system as though better mobility causes a decreas of blood pressure and lowering of heart rate.

Flexibility increases, as well as coordination and strength. It also leads to increased self-esteem, decreased depression and improved socialization.²²

Physical activity reduces the metabolic risk factors (improving lipid profile, reducing hyperinsulinemia, improving insulin action and glucose tolerance). Physical activity that increases endurance is recommended, rather than one that increases the power, and priority is given to activities carried out in the open air. Types of physical activities that are recommended are swimming, biking, rowing, skiing, dancing and walking. Among them a walk is in the first place, so that we can, by using UKK test, to recommend frequency, intensity and duration of these activities in a simple way. Because of the inverse correlation between the values of VO-2max and BMI, it is necessary to maintain body mass or BMI in the optimal range 18.5 - 24.9, due to its impact on cardiovascular capacity.²³

Conclusion

Between the values of VO2max and BMI, there is an inverse correlation (an increase in one variable, decreases the other variable), or, decreasing BMI values are increasing values of VO2max and vice versa. Because of the inverse correlation between the values of VO2max and BMI, body mass (or BMI) is necessary to maintain in the optimal range 18.5 - 24.9, due to its impact on cardiovascular capacity. Poor aerobic capacity in young people, who have a tendency to weight gain, can cause cardiovascular comorbidity in middle age of life.

References

- 1. Ivković-Lazar T. Gojaznost, univerzitetska naučna knjiga, Medicinski fakultet Univerziteta u Novom Sadu 2004; 112-82.
- 2. Canadian guidelines for body weight classification in adults : applicatotion in clinical practice to screen for overweight and obesity and to assess disease risk, JAMC 2005; 172(8):995-8.
- 3. Janssen I, Katzmarzyk PT, Ross R. Body mass index , waist circumference, and health risk: evidence in support of current National Institutes of Health guidelines. Arch Intern Med 2002; 162: 2074-9.
- Allen DT . Why BMI ? J Ky Med Assoc 2004; 102 (4):171-5.

- 5. Schindler K , Ludvik B . Methodische und praktische Aspekte der Bestimmung der Körperzusammensetzung , Wien Med Wochenchr 2004; 154 (13-14):305-12.
- 6. Wechsler JG . Sinnvolle diagnostic der adipositas , Wien Med Wochenchr 1998;148:393-6.
- 7. Liberopoulos EN, Mikhailidis DR, Elsaf MS. Diagnosis and management of the metabolic syndrome in obesity. Obesity Reviews 2005; 6(4):283-96.
- 8. Basset FA, Boulay MR. Specificity of treadmill and cycle ergometer tests in triathletes, runners and cyclists. Eur J Appl Physiol 2000; 81:214-21
- Noonan V, Dean E. Submaximal exercise testing: Clinical application and interpretation. Phys Ther. 2000; 80:782-807.
- Montoye HJ, Ayen T, Washburn RA. The estimation of Vo2max from maximal and submaximal measurements in males, age 10–39. Res Q. 1986; 57:250 –3.
- 11. American College of Sports Medicine. Guidelines for Exercise Testing and Prescription. 5th ed. Philadelphia, Pa: Lea & Febiger; 1995.
- 12. Laukkanen R, Development and Evaluation of a 2-km Walking Test for Assessing Maximal Aerobic Power of Adults in Field Conditions. Doctoral Dissertation. Kuopio University, Publications D. Medical Sciences 23, Kuopio 1993.
- 13. Laukkanen R, Hynninen E (ed). Guide for the UKK Institute 2-km Walking Test. UKK Institute, 5th rev. ed., Tampere 1997.
- Laukkanen R, Kukkonen-Harjula K, Oja P, Rasanen M, Vuori I. Prediction of change in maximal aerobic power by the 2-km Walk Test after walking training in middle-aged adults. Int J Sports Med 2000; 20: 113–116.
- 15. Laxmi C.C. A Study of effect of body mass index cardiorespiratory fitness in young males Department of Physiology Sri Devaraj Urs Medical College Tamaka, Kolar, 2008.
- 16. Thang SH, Naveed S, Mike L. Assessment of obesity and its clinical implications.BMJ 2006; 333: 695-8.
- 17. Wilmore JH, Costil DL. Physiology of Sport and Exercise: 3rd edition. Champaign, IL: Human Kinetics. 2005.
- Peter Pribis, Carol A. Burtnack, Sonya O. McKenzie and Jerome Thayer. Trends in Body Fat, Body Mass Index and Physical Fitness Among Male and Female College Students. Nutrients 2010; 2, 1075-85

- 19. Lowry, R., Galuska, D.A., Fluton, J.E., Wechsler, H., Kann, L., Collins, J.L. Physical activity, food, choice, and weight management goals and practices among US college students. Am. J. Prev. Med. 2008; 18, 18-27
- 20. Mestek, M.L., Paisance, E., Grandjean, P. The relationship between pedometer determined and self-reported physical activity and body composition variables in college-aged men and women. J. Am. Coll. Health 2008; 57, 39-44.
- 21. McArthur, L.H, Raedeke, T.D. Race and sex in college students physical activity correlates. Am. J. Health Behav. 2009; 33, 80-90.
- 22. Taliaferro, L.A., Rienzo, B.A., Pigg, R.M., Miller, M.D., Dodd, V.J. Association between physical activity and reduced rates of hopelessness, depression, and suicidal behavior among college students. J. Am. Coll. Health 2008; 57, 427-435.
- 23. Fossum E, Hoieggen A, Moan A, Rostrup M, Kjeldsen: Insulin sensitivity is related to physical fitness and exercise blood pressure to structural vascular properties in young men. Hyperten 1999; 33: 781-786.

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Data mining analysis of prescripted drugs time series

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Abstract

Background: There is annually a large number of prescripted drugs within medical institutions. Inadequate drug prescription leads to drug overuse per a patient and to unnecessary financial costs. By analysing the the content and the number of prescripted drug packets, information related to the prescription of a drug groups in a certain time period can be obtained. Collected information could be used for resource management and reduction of costs in a medical institution, as well as for improvement of a general health condition of the population.

Material and Methods: Research material has been taken from four-year records of General Health practice and contains data on prescribed drugs. We examined the time series of prescribed drug packages divide into groups according to ATC classification. The correlations between individual drugs as well as between groups of drugs have been explored. Drug grouping per dynamics of prescription has been performed by applying data mining upon time series.

Results: During the prescription of different drugs, both individual and group ones,a mutual connection has been determined over an observed time interval. Three levels of ATC classification groups have been determined to have a high degree of correlation in prescription of drugs from the same group in the consecutive years. Also, during the observed time period a pairs of drug groups with significant correlation have been determined. By analysing time series, a high increase has been noticed since September 2009 in drug prescription for the drug group of respiratory system and nervous system, compared to the expected values. Further cluster analysis shows the reasons for the origin of this rise in prescriptions that are retained until present days.

Conclusion: Based on correlations and found drug separation into clusters, the useful informati-

on has been collected about drug prescription. Since data used in the research has not been specific for the observed medical institution, the methods used could be applied to data from other medical institutions. All gained information can contribute to better organisation of drug supply with the aim to keep drug reserves in an optimal level, and also help in the process of determination of an essential drug list.

Key words: Resource administration, medical information systems, time series, clustering.

Introduction

The main goal of modern clinical pharmacology, particularly studies that address the research drug usage, is to promote their rational use. Each medical institution collects and keeps data on prescriptions being used for regulation of payment from Republic Fund of Health Insurance. Collected data can be used as a source for very useful information that can be used to improve the efficiency of customer service, better approach to patient treatment and diagnosis, and more efficient administration of a medical institution. This information is not always possible to produce by using only conventional statistical methods, their extraction data mining methods should be used as well [1, 2, 3].

Drug management includes all aspects of therapeutic drug use, from a health institution to a patient as an individual. Good management of drugs secures resource saving (financial, time/people and equipment/drugs), and the effectiveness of the health system to the level of the patient. In Serbia, based on RZZO data [4] in the period from 2009 to 2010, the annual average of 88,025,110 drug packets were prescribed to 60,205,863 prescriptions. In line with this, there was a increase of health insurances comparing 2010 and 2009 (2.72%), and also a number of prescribed drug packets (4.72%). These percentages may be an indicator that the drugs have not been used as they should, and they represent a strong argument for research of prescribed drugs.

In this paper, the number of prescribed drug packets have been analysed by observing time series of issued prescriptions. The aim of this analyse has been to determine a drug groups that have the same changes in the quantity of prescribed packets. The analysis result could be used for planning of procurement of these drug groups to maintain stocks of drugs in a pharmacy. The analyse has been performed both for the individual and groups of drugs. Since there is a large number of available drugs on the market, drug groups have been observed pursuant to ATC (Anatomical Therapeutic Chemical) [5] classification. In the ATC classification system, the active substances are divided into different groups at five different levels according to the organ or system on which they act (1st level) and their therapeutic (2nd level), pharmacological (3rd and 4th levels) and chemical properties (5th level). The national JKL classification of drugs has been also used in the research, in order to have more precise analyse related to this pharmaceutical form of a drug [6].

Material and methods

A collection of data includes the records of prescribed drugs in the General Health practice in the period from 10.1.2007 to 7.4.2011. Each record contains drug name, its manufacturer, ATC and JKL code, doses and number of prescribed packets. In order to protect privacy of patients and doctors, the material does not include data on their names or other types of identification (number of a health insurance, doctor's code, etc.).

Since a drug supply is being performed on weekly basis, the initial data has been aggregated. During aggregation, there has been no difference among pharmaceutical equivalent drugs. Two drugs are pharmaceutical equivalent if they have the same active substance in the same hemical form, the same quantity/concentration of the active substance and the same pharmaceutical form [7]. After aggregation, the collected set contained 449 drugs. Aggregated data has been placed in relational database (we used IBM DB2) tables due to simpler approach and mining.

Collected data represents values which can be ordered in time, being suitable for time series analysis. Because of large variations in data values, it was necessary to make normalization in order to have a successful flow of the series, i.e. transformations leading to time series levelling. The transformations have been made with 9-Hendersen movable averages for time series levelling or a logarithm transformation for data normalization [8]. The similarity of observed drugs can be found by time series clustering [9, 10]. As a measure for similarity of cluster elements (1) a distance of time series, or (2) a correlation of time series has been used. The Euclidean distance has been used in the paper as a measure for determination of time series distances. Since the Euclidean distance has been sensitive onto noises and aggregation exceptions, the number of values and noises has been reduced by logarithm transformation. The Pearson's correlation coefficient (kk) has been used as a measure for determine correlation of two time series. The distance D of two time series has been determined by a formula ([9]):

$$D^2 = 2 * (1 - kk)$$
 (1)

For comparison of results of different clustering methods, it has been used "silhouette" coefficient [1]. Let A determine an average distance from elements of the same cluster, where B has been a minimal distance from elements from other clusters. Then, the silhouette coefficient for a certain element has been calculated by a formula

$$S = 1 - \frac{A}{B} \quad (2)$$

The silhouette coefficient for a cluster has been calculated as the average value of coefficients for each element of the cluster, while the coefficient for entire clustering has been calculated as the average of values of coefficients of all elements of all clusters. The algorithm realizing these methods was implemented in the programming language C. For the analyzing the time series and their visual presentation, IBM Infosphere Intelligent Miner [12] was used.

Results and discussions

Time series of total prescriptions per days for 2008, 2009 and 2010 has been shown on Figure

1. It is noticeable that the number of prescribed drugs constantly grows from year to year. A number of exceptions can be observed in the second half of a year as a consequence of holidays. We have a similar situation with graphics showing time series of anatomic drug groups or individual ones. The most prescribed drugs in the period from 2008-2010 are shown in the Figure 2. There has been no strict trend of prescribed drug packets increase per years. Due to the rise of population, the rise of total number of prescribed drug packets was expected. This result indicates the need for analyzing the dynamics of drug prescribing.

The analysis of time series of drug prescriptions in 2008, 2009 and 2010. for each anatomic group (first level of ATC classification) have been performed. It has been noted that drugs from the J group have clearly emphasised season for all three years, being a consequence of more frequent usage in winter months. The drugs of M, C, H and S groups neither had an emphasis season, nor had shown significant exceptions or rise trend in observed period. The drugs of G group have had a permanent rise trend in all three years, whereas regarding the drugs of B group a permanent rise trend has been noticed in the last year, which was different in comparison with the previous two years where the trend in the first half of the year was negative (data not shown). Drugs of R and N groups have had a sharp rise in September 2009,



Figure 1. Number of daily prescribed drug packets for the years 2008-2010



Figure 3. Continuous line- time series of number of prescripted drug packets per weeks for the years 2008-2010 ATC-R, dashed lines- the number of prescripted drug packets per a month for 2009 and 2010, in the entire Serbia (numeric values reduced 1000 times)

with keeping reached values of irrelevant variations in 2010 (Figures 3 and 4). Regular behaviour of the drugs of other groups (P, L, D) which have high values variations could not be observed. . For all other groups time series analyses of prescribed drugs in therapeutic groups and in significant individual drugs have been performed. The results will be illustrated on the example of R and N groups. A possible explanation for a sharp increase in a number of prescript drugs of R group in September 2009 could be the beginning of a school year and announced epidemic of H1N1 flu. However, there has been no explanation for the rise in prescription of drugs in this group through the whole 2010. Different from the observed material, in the entire territory of Serbia, the drugs of this group had a rise in September 2009, but the number of prescripted drugs in the first half of 2010 fell to the level of the first half in 2009. The drugs of N group have similar behaviours in the observed material, while in the entire territory of Serbia drugs of group N have similar behaviours in 2009 and 2010, with a slight increase and without major oscillations (Figures 3, 4).

Figures 5 and 6 shows the behaviour of the therapeutic subgroups within ATC groups N and R whose number of prescribed drug packets is statistically significant. A number of prescribed drug packets of all therapeutical subgroups of N group have the same behaviour as whole group N.



Figure 2. The total number of prescribed drug packets in 2008, 2009 and 2010, being the most prescribed ones



Figure 4. Continuous line- time series of number of prescripted drug packets per weeks for the years 2008-2010 ATC-R, dashed lines- the number of prescripted drug packets per a month for 2009 and 2010, in the entire Serbia (numeric values reduced 1000 times)

Within R group, the growth has been discovered only within subgroup R03, while all other sub-groups remain more or less at constant level. It had been found that that majority of individual drugs of N group had an increasing trend (see further text about clustering time series), while within R03 subgroup, numbers of prescribed individual drugs had increased and fallen in different moments (Figure 7). In order to find a connection in prescription of the same drug in the consecutive years and connection in prescription of two drugs in observed period, a correlation analyse of time series has been performed. The correlation coefficient has been calculated for each anatomic group of drugs upon time series of the number of prescribed drug packets, observed in two time periods of equal length, with a one year difference in the starting period. In the first part of Table 1 are shown only those anatomic groups having a significant correlation. In the same way, time series of prescribed drug packets have been compared in the consecutive years upon therapeutic drug equivalents and upon pharmaceutical equivalents, where results have been also shown in Table 1(2nd part). Since pharmaceutical equivalency requires equivalence of the pharmaceutical form of drugs, a set of observed drugs has been narrowed to drugs whose pharmaceutical form belong to group 1 of first level JKL classification (dragees, capsules, lingualletes, lozenges, tablets, effervescent tablets), since other forms occurs rarely. Also, the drugs whose total number of prescription is less than 1000 in the observed period have not been included, because of high occurrences of zeros in the time series lead to significant correlations. Further analyses are applied to the remaining group of 128 pharmaceutically equivalent drugs.

Similarities of drugs or group of drugs in the consecutive years, based on obtained coefficients of correlation, have been shown (Table 1, 3rd part). Being similar in the consecutive years means in the same period of the year they have gained their extremes, had similar seasons and such a permanent trend. This information could be applied in planning of drug supply. The number of these



Figure 5. The review of time series of the number of prescribed drug packets from 2008 to 6. 2011, observed in therapeutic drug subgroups N04,N05,N06



Figure 6. The review of time series of the number of prescribed drug packets from 2008 to 6. 2011, observed in therapeutic drug subgroups R03,R06



Figure 7. The review of time series of the number of prescribed drug packets in the period from 2007 until 2011, observed per drugs blue -RO-3DA04, red -R03DA05 in Intelligent miner

Correlation for drugs influencing the same anatomic group		Correlation equiva	for therapeutic lent drugs	Correlation for pharmaceutical equivalent drugs	
Anatomic group	Correlation coefficient	Therapeutic group	Correlation coefficient	ATC	Correlation coefficient
J	0.537371	A10	0.65	A10BA02	0.80326
N	0.508214	J01	0.55	N05BA06	0.707757
R	0.503797	G04	0.52	C09BA08	0.666283
G	0.492137	N06	0.49	N05BA08	0.550783
Н	0.403917	M05	0.43	N03AE01	0.532469
		N05	0.42	C09BA09	0.519971

Table 1. Similarity of drugs or group of drugs in the consecutive years

ATC drug 1	ATC drug 2	Correlation coefficient
C01DX12	C09BA09	0.93
A10BA02	C09BA08	0.92
A10BA02	N05BA06	0.91
C01DX12	C07AB07	0.90
C01DX12	N05BA06	0.90
N03AE01	N05BA06	0.90
N05BA06	N06AB03	0.90
A10BA02	G04CA02	0.89
N03AE01	N05BA06	0.89

Table 2. The most correlated time series in prescription of different drugs

drugs has been small and majority of drugs have not had any significant connection in prescription in the consecutive years.

To get higher quality analysis, pairs of drugs with similar trends in prescription have been found. By correlation analysis of time series of different drugs, 165 pairs of drugs have been found with correlation coefficient higher than 0.8, and additional 993 pairs that have correlation coefficient between 0.6 and 0.8. The most correlating drugs are shown in Table 2.

Additional quality in analysing of drug prescriptions and detection of individual connections in the prescription of drugs has been achieved by grouping their trends and quantities. For discovering the time series groups of drug prescriptions, clustering has been used as one of the technique of data mining. Since the exact number of clusters is not known in advance, a hierarchical agglomerative algorithm of clustering for grouping of time series has been the most appropriate one. Distance among clusters was calculated as the average distance of pair elements. For clustering quality evaluation the silhouette coefficient was used. For a comparison of two time series, the Pearson's correlation coefficient was used as a part of the measure. (see Formula 1). The tree level in which all elements within the clusters satisfy the condition that the other elements in the cluster are correlated with a coefficient greater than 0.5 is taken as a result of clustering.

The layout of hierarchical tree with fifteen level and the discovered clusters is shown in Figures 8 and 9. In the fifteenth level a 67 cluster exists,



Figure 8. Clusters in levels and correlations in the ones

out of which 54 are single-members (one drug in cluster) and remaining 13 are multi-member (with total 74 drugs). The drugs which belonged to single-member clusters are not shown. Further merging of clusters has not been appropriate since it has weakened the correlation of elements within the clusters. The obtained clusters represent drug groups having similar dynamics of prescribing, similar trend and seasons. In specific clusters, time series can have one of the following characteristics:

- A permanent rise trend for the entire observation period
- A rise until the half of the observed period and then a sharp fall
- A sharp increase in one period is retained until the end of the period
- Season variation during the entire period
- A rise trend and season variations.

The clusters contain a large number of drugs from same anatomic, therapeutic group or pharmaceutically equivalent drugs. A number of drugs from group N are grouped together into cluster characterised by sharp rise in prescription in September 2009. Different drug doses with ATC code RO3DA04 have been separated into a special cluster, whose time series had a sharp rise at the end of 2009, and in the middle of 2010 they returned to the initial level. Some drugs from C anatomic group, representing the most frequently used drugs, have been grouped to the same cluster. Antibiotics (group J) were grouped in a cluster (in this cluster is a drug M03BX07 which is from another anatomic group). It has been noticed that some pairs of drugs have a high Pearson's correlation coefficient, but don't belong to the same anatomic group (A10BA02, C09BA08 with kk = 0.9).

These results are typical for the geographical area from which the sample was taken and information about the increase/decrease of the number of patients suffering from an illness or a change in therapy for certain disease can be drawn from them. One of the interesting direction of research could be whether the same drug has been prescribed with a similar dynamics in different concentrations, or, in one moment, there has been one dominant concentration. Clustering with metrics presented in Formula 1. has not been sensitive to the difference in the number of prescribed drug packets per week, but only to changes in prescription trend. The obtained clusters contain drugs whose total number of boxes of prescription varies widely. When the clustering uses Euclidean distance as a measure between time series, total number of prescribed drug has a greater influence on the clusters generation. Applying this measure, we have gained drug groups whose connection can be interpreted as:

- Therapeutical dependence, for instance, two drugs are often used as a combination in a disease treatment,
- A large number of patients who have multiple diagnoses, use as a therapy specific combination of drugs, which are note associated therapeutically,



Figure 9. Clusters in levels and correlation in the ones (cont. from Figure 8)

- Simultaneous increase or fall of a number of patients from a group of diseases.

Alignment of time series is performed by means of transformation Henderson's moving average. For the easier comparison of drug being prescribed in different quantities, data normalisation has been performed by logarithm transformation. Results of clustering the same material (related to 128 pharmaceutically equivalent drugs) with Euclidian distance as a measure are shown on Figure 10. This measure produce clusters with drugs which have similar total quantity of prescribed packets.

Clusters 10 and 11 contain the most frequently prescribed drugs. In the previous clustering with a metrics based on correlation (Formula 1), these drugs belong to the same cluster, except two drugs which are placed in the cluster whoes elements have had extremely rise trend. The drugs whose



Figure 10. Clusters gained by hierarchy clustering



Figure 11. Hierarchy AVG clustering- the coefficient correlation measure, drugs influencing the nerve system. Five multi-member clusters produced



Figure 12. Hierarchy AVG clustering-the Euclidean distance measure, drugs influencing the nerve system. Six multi-member clusters produced.

time series have shown a huge rise trend belongs to only one cluster, similarly as in previous clustering. The results of both clusterings are the best to be used in a combination, because only when they are joined they can give a clearer image on drug prescription. While analyzing at both clusterings, a group of drugs was noticed coming from different anatomic groups but belonging to the same cluster in both clustering (N05BA01 ,M01AC06 ,N05BA12 ,A10BA02 ,C09BA09 ,N05BA06).

Clustering with two previously mentioned distance measures have been preformed on each anatomic drugs group separately. Figures 11. and 12. contain, as an example, the results for N group. From the results it can be observed that the group N contains a number of drugs (located in cluster 0) which had a sharp increase of the number of prescriptions prescribed in the second half of the observation period.

Clusters 6 and 7 (Figure 12) contain drugs that are prescribed frequently. As these drugs are not grouped in cluster 0 (grouped with correlation) we can conclude that these drugs have not had a sharp rise, as well as that drugs from cluster 0 are not prescribed frequently. A sharp rise of number of prescribed drugs in the group N (Figure 4) is a consequence of drugs that are not prescribed in large quantities.

Conclusion

Health is one of the areas where data mining has not been adequately applied for the analyses of data. In this paper, it has been shown that by the use of data mining methods and tools, the results of the analysis could be significantly improved in relation to traditional statistical methods. Drug groups, tightly connected either per prescription quantity or prescription dynamics, have been defined applying clustering upon time series of prescribed drug packets. Some specific drug groups have been found, whose prescription has been exceptional compared to most of other drug groups, and further analyse has shown the content and the reasons for groups R and N.

Based on produced results, it can be determined concrete actions to be applied in the aim of drug usage reduction, their adequate application, and drug supply planning. Also, the time and scope of taking preventive actions could be predicted in order to improve general health of the population.

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Reference

- 1. Tan, P-N., M. Steinbach, V. Kumar. Introduction to Data Mining, Pearson Education, (2006).
- 2. Krzysztof J. Cios, Medical Data Mining and Knowledge Discovery, Studies in Fuzziness and Soft Computing,(2001)
- 3. Michael Berry, Murray Browne, Lecture notes in data mining, UNIVERSITY OF TENNESSEE, USA(2006)
- 4. Republic Institute for health insurance, www.rfzo.rs
- WHO Collaborating Centre for Drug Statistics Metodology http://www.whocc.no/atc/structure_and_principles/
- 6. National Drug register /Nacionalni registar lekova,Agencija za lekove I medicinska sredstva Srbije,ISSN 1452-3337,(2012)
- 7. Lj.Tasić,D.Krajnović,M.Petrić et al., Farmaceutska praksa/ Praktikum, Univerzitet u Beogradu, Farmaceutski fakultet,(2010)
- 8. Zlatko J. Kovačić: Analiza vremenskih serija ,Univerzitet u Beogradu,Ekonomski fakultet (1995)
- 9. Warren Liao ,Clustering of time series data—a survey,Patern Recognition 38 (2005) 1857-1874
- 10. S.Hirano, TTsumoto, Empirical Comparison of Clustering Methods for Long Time-Series Databases, Springer-Verlag Berlin Heidelberg 2005
- 11. Chu, S., Keogh, E., Hart, D., Pazzani, M. (2002). Iterative Deepening Dynamic Time Warping for Time Series. In proceedings of the second SIAM International Conference on Data Mining.
- 12. IBM, Mining in the Design Studio, SC19-1259-01

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Athletes' heart syndrome in University of Sarajevo students

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Abstract

Athletes' heart syndrome, (AHS) also known as athlete's heart or athletic bradycardia, is a nonpathological condition commonly seen in sports medicine, in which the human heart is enlarged, and the resting pulse lowers, due to significant amounts of exercise, commonly aerobic exercise, and especially in endurance sports. Athlete's Heart is common in athletes who routinely exercise more than an hour a day, and appears occasionally among heavy weight trainers. The condition is generally believed to be a benign, but may sometimes be hard to distinguish from other serious medical conditions. For example, the results of medical tests such as an electrocardiogram can be mixed up with those of a serious heart disease by mistake. Athlete's Heart is a result of dynamic physical activity such as (more than 5 hours a week) aerobic training or tennis, rather than static training such as weight lifting. During intensive prolonged endurance or strength training, the body signals the heart to pump more blood through the body to counteract the oxygen deficit building in the skeletal muscles. Enlargement of the heart is a natural physical adaptation of the body to deal with the high pressures and large amounts of blood that can affect the heart during these periods of time. Over time, the body will increase both the chamber size of the left ventricle, and the muscle mass and wall thickness of the heart. Cardiac output, the amount of blood that leaves the heart in a given time period (i.e. liters per minute), is proportional to both the chamber sizes of the heart and the rate at which the heart beats. With a larger left ventricle, the heart rate can decrease and still maintain a level of cardiac output necessary for the body. Therefore, it is very common for athletes with AHS to have lower resting heart rates than nonathletes.

Objective: To investigate the presence and characteristics of the athletes' heart syndrome in the University of Sarajevo students.

Materials and method: The study was prospective, retrospective, clinical, and descriptive analytical. The sample consisted of students at the University of Sarajevo. The sample included students in regular and systematic examinations of the Institute of Public Health for students at the University of Sarajevo in the period 2007 - 2010. Methods of research included personal history, electrocardiogram, 24h-Holter monitoring of the heart and echocardiogram.

Results: Athletes' heart was documented in 16 boys (41%), 2 girls (2.86%): total of 18 patients (16.51%). Following heart rhythm disturbances were recorded in athletes' heart: bradycardia (95%), tachycardia (86 %); tachyarrhythmia extrasystolica ventricular et sup. in 11 patients (10.09%), atrial fibrillation in 2 boys (1.83%), RBBB in 16 patients with athletes' heart (14.68%), ST elevation was recorded in 23 patients, or (21.10%), out of that in 16 boys or (41.02%) and 7 girls, or (10.00%), ST depression was recorded in 21 patients, or (19.26%) out of which 6 boys or (15.38%) and 15 girls, or (21.42%), PQ interval was shortened in 1 patient, or (0.92%), prolonged QT interval in 7 patients, or 6. 42%, nodal rhythm and in 3 patients (2.75%) Torsade de pointes in a patient or 0.92%, AV block in 9 patients, or (8:26%). Dilated cardiomyopathy was recorded in 6 patients, or (5.50%); hypertrophic cardiomyopathy in 2 patients, or (1.83%).

Key words: University of Sarajevo students, sports activities, athletes' heart syndrome, cardiac rhythm disorders, hypertrophic and dilated cardiomyopathy.

Introduction

Athletic heart syndrome, (AHS) also known as athlete's heart or athletic bradycardia, is a nonpathological condition commonly seen in sports medicine, in which the human heart is enlarged, and the resting pulse lowers, due to significant amounts of exercise, commonly aerobic exercise, and especially in endurance sports. Athlete's heart is common in athletes who routinely exercise more than an hour a day, and appears occasionally among heavy weight trainers. The condition is generally believed to be a benign, but may sometimes be hard to distinguish from other serious medical conditions. For example, the results of medical tests such as an electrocardiogram (ECG) can be mixed up with those of a serious heart disease by mistake.

Description

Athlete's Heart is a result of dynamic physical activity such as (more than 5 hours a week) aerobic training or tennis, rather than static training such as weight lifting. During intensive prolonged endurance or strength training, the body signals the heart to pump more blood through the body to counteract the oxygen deficit building in the skeletal muscles. Enlargement of the heart is a natural physical adaptation of the body to deal with the high pressures and large amounts of blood that can affect the heart during these periods of time. Over time, the body will increase both the chamber size of the left ventricle, and the muscle mass and wall thickness of the heart. Cardiac output, the amount of blood that leaves the heart in a given time period (i.e. liters per minute), is proportional to both the chamber sizes of the heart and the rate at which the heart beats.

With a larger left ventricle, the heart rate can decrease and still maintain a level of cardiac output necessary for the body. Therefore, it is very common for athletes with AHS to have lower resting heart rates than nonathletes. The heart becomes enlarged, or hypertrophic, due to intense cardiovascular workouts, creating an increase in stroke volume, an enlarged left ventricle (and right ventricle), and a decrease in resting pulse along with irregular rhythms. The wall of the left ventricle increases in size by about 15-20% of its normal capacity. There is no decrease of the diastolic function of the left ventricle. The patient may also experience an irregular heartbeat and a resting pulse rate between 40-70 beats per minute, also known as bradycardia. The level of physical activity in a person determines what physiological changes the heart makes. There are two types of exercise: Static (strength-training) and dynamic (endurance-training). Static exercise consists of weight lifting and is mostly anaerobic, meaning the body does not rely on oxygen for performance. It also moderately increases heart rate and stroke volume (oxygen debt). Dynamic exercises are running, swimming, skiing, and cycling, which rely on oxygen from the body. This type of exercise also increases both heart rate and stroke volume of the heart. Both static and dynamic exercises involve the thickening of the left ventricular wall due to increased cardiac output, which leads to physiologic hypertrophy of the heart. It has been shown that once athletes stop training, the heart returns to its normal size.

History

The athlete's heart was first described in 1899 by S. Henschen. He compared the heart size of cross-country skiers to those who lived sedentary lives. He noticed that those who participated in competitive sports displayed symptoms of Athlete's Heart Syndrome. Henschen believed the symptoms were a normal adjustment to exercise, and felt there was no need for concern. Henschen believed that the entire heart became enlarged, when in fact it is only the left side that becomes hypertrophic. He also believed athletes with AHS lived shorter lives than those who did not acquire the syndrome. Because his research occurred throughout the 19th century, technology was limited, and it became difficult to come up with appropriate ways to measure the hearts of athletes. Few believed in Henschen's theory about athletes having larger hearts than those who did not participate in sports. Today, Henschen's original theory has proved to be correct.

Symptoms

Athlete's Heart most often does not have any physical symptoms, although an indicator would be a consistently low resting heart rate. In most cases, athletes do not realize they even have the syndrome until they go for their annual physical examination. The reason behind the "mystery" as to why there often are no symptoms when one's heart is physically enlarged is because Athlete's Heart is an adaptation of the body to the stresses of physical conditioning and aerobic exercise. When one is diagnosed with Athlete's Heart, there are usually three characteristics that accompany the condition that would indicate a heart condition if they were seen in a non-athlete: bradycardia, cardiomegaly, and cardiac hypertrophy.

Bradycardia is a slower than normal heartbeat around 40-60 beats per minute. Cardiomegaly is the state of an enlarged heart. Cardiac hypertrophy is the thickening of the muscular wall of the heart, specifically the left ventricle, which pumps oxygenated blood to the aorta. In highly trained athletes' bodies, more blood and oxygen is required to the peripheral tissues of the arms and legs. A larger heart results in higher cardiac output, or, in other words, more blood is being pumped out with each beat. With high cardiac output, the heart can allow itself to beat less, thus the bradycardia occurs. Another sign of Athlete's Heart Syndrome is an S3 Gallup, which can be heard through a stethoscope. This sound can be heard as an irregularly shaped heart is filling with blood. The diastolic pressure creates a disordered flow of blood into the heart. However, if an S4 Gallup is heard, the patient should be given immediate attention.

An S4 Gallup is a stronger and louder sound created by the heart if it is diseased in some way. It is typically a sign that there is a serious heart condition present in the athlete. According to recent research using imaging equipment capable of imaging the heart during exercise, the stroke volume of hearts of top athletes increases during exercise. This effect is absent in recreational athletes.

Diagnosis

AHS is usually diagnosed during a routine screening or during tests for other medical issues. An enlarged heart can be seen on an echocardiogram or sometimes a chest X-ray. Due to the similarities between AHS and more serious cardiac problems, an electrocardiogram (ECG) and exercise stress echo tests are sometimes performed. AHS and left ventricular hypertrophy are usually indistinguishable via EKG, however LVH is usually dismissed in the young and fit. The EKG can detect sinus bradycardia, a resting heart rate of fewer than 60 beats per minute. This is often accompanied by sinus arrhythmia. The heartbeat of a person with AHS can sometimes be irregular while at rest, but usually returns to normal after exercise begins. One common cardiovascular disease that has similar EKG reads is hypertrophic cardiomyopathy, which is characterized by the thickening of the heart's walls. This genetic disorder is found in 1 out of 500 Americans and is responsible for thousands of sudden death cases every year. Of all sudden death cases, only about 8% are exerciserelated. The Table 1. shows distinguishing characteristics of the two conditions.

The medical history of the patient (endurances sports) and physical examination (bradycardia, and maybe a third or fourth heart sound) can give important hints.

- Electrocardiography (EKG) - typical findings in resting position are for example sinus bradycardia, atrioventricular block (I° and II°) and right bundle branch block - all those findings normalize during exercise

Feature	Athletic Heart Syn- drome	Cardiomyopathy
Left ventricular hypertrophy	< 13 mm	> 15 mm
Left ventricular end-diastolic diameter	< 60 mm	> 70 mm
Diastolic function	Normal (E:A ratio > 1)	Abnormal (E:A ratio < 1)
Septal hypertrophy	Symmetric	Asymmetric (in hypertrophic cardiomyopathy)
Family history	None	May be present
BP response to exercise	Normal	Normal or reduced systolic BP response
Deconditioning	Left ventricular hyper- trophy regression	No left ventricular hypertrophy regression

Table 1. Features Distinguishing Athletic Heart Syndrome From Cardiomyopathy

- X-ray examination of the chest shows an increased heart size
- Echo cardiogram the differentiation between physiological an pathological increase of the heart's size is possible especially by estimating the mass of the wall (not over 130 g/m2) and its end diastolic diameter (not much less 60 mm) of the left ventricle.

Prognosis

Although the heart experiences structural changes (i.e. wall thickening) that are common with some cardiac disease, no unfavorable effects are evident. 80% of people affected by this syndrome show a decrease in such structural changes and in bradycardia with detraining. Unfortunately, the lack of long-term data limits the ability to determine whether or not the remaining 20% of people who do indeed have residual chamber enlargement have experienced any negative side effects. Athlete's Heart is not dangerous for athletes, but if a non-athlete has the symptoms of bradycardia, cardiomegaly, and cardiac hypertrophy, another possible illness is present. It is very important not to confuse Athlete's Heart with the reason that some athletes have Sudden Unexpected Death (SUD) during or shortly after a workout. In many cases there are no signs that an athlete will experience SUD, but it is always due to an underlying heart disease and not Athlete's Heart. Risks of Athletic Heart Syndrome. Low resting heart rate in athletes is common hence many times, a dangerously low rate may go undiagnosed and delay in treatment may result in sudden unexpected death (SUD). SUD usually happens during or after any physical activity. In most cases of SUD, a structural defect in the heart is the reason behind the death. Hence, it is very important to get the symptoms minutely observed as they can resemble with other heart related diseases.

Though SUD takes place rarely, it is more common in the people above 35 years of age. Careful examination of resting heart rate chart of athletes and timely treatment is the only solution to avoid such situations. Sports and other physical activities are good for health and hence, should not be discontinued. They should be done in moderation. There is as such no cure for this syndrome. It may be typically treated by deconditioning the athlete from his physical exercise routine for three months which will bring back his heart to its normal size. You need to take care of yourself and your heart and get it checked at regular intervals to avoid complications.

Treatment

No treatment is required for people with athletic heart syndrome. Athlete's Heart does not pose any physical threats to the athlete, and there have been no negative long term effects tied to Athlete's Heart. It is recommended that the athlete see a physician and receive a clearance to be sure that the symptoms are due to Athlete's Heart and not another heart disease, such as cardiomyopathy. If the athlete is uncomfortable with having Athlete's Heart or a differential diagnosis is difficult, typical treatment is deconditioning the athlete from exercise for a period of three months. During this time, the heart will return to its regular size. This deconditioning is usually met with resistance since it is changing the athlete's lifestyle. The only risky aspect of Athlete's Heart is if an athlete or non-athlete simply assumes that they have the condition, instead of making sure it is not a life-threatening heart illness

Due to the fact that several well-known and high profile cases of athletes experiencing sudden unexpected death due to cardiac arrest (Reggie White, Wes Leonard), there is a growing movement to make an effort to have both professional and school-based athletes screened for cardiac and other related conditions, usually through a careful medical and health history, a good family history, a comprehensive physical examination including auscultation of heart and lung sounds and recording of vital signs such as heart rate and blood pressure, and increasingly, for better efforts at detection, such as an electrocardiogram. An electrocardiogram (ECG) is a relatively low-cost procedure to administer and interpret, compared to more invasive or sophisticated tests; it can reveal or hint at many circulatory disorders and arrhythmias. Part of the cost of an ECG may be covered by some insurance companies, though routine use of ECGs or other similar procedures such as echocardiography (ECHO) are still not considered routine in these contexts. Widespread routine ECGs for all

potential athletes during initial screening and then during the yearly physical assessment could well be too expensive to implement on a wide scale, especially in the face of the potentially very large demand. In some places, there is a shortage of funds, portable ECG machines, or qualified personnel to administer and interpret them (medical technicians, paramedics, nurses trained in cardiac monitoring, advanced practice nurses or nurse practitioners, physician assistants, and physicians in internal or family medicine or in some area of cardiopulmonary medicine).

If sudden cardiac death occurs, it is usually because of pathological hypertrophic enlargement of the heart that went undetected or was incorrectly attributed to the benign "athletic" cases. Among the many alternative causes are: episodes of isolated arrhythmias which degenerated into lethal VF and asystole, and various unnoticed, possibly asymptomatic cardiac congenital defects of the vessels, chambers, or valves of the heart. Other causes include: carditis, endocarditis, myocarditis, and pericarditis whose symptoms were slight or ignored, or were asymptomatic. The normal treatments for episodes due to the pathological lookalikes are the same mainstays for any other episode of cardiac arrest: immediate activation of the emergency response system, CPR (approximately 100 beats per minute; respirations may or may not be given, depending on the level of training), automatic defibrillation in an effort at cardioversion and restoration of normal sinus rhythm, and if trained, administration of intravenous epinephrine or amiodarone (if not contraindicated, and only if initial defibrillation efforts fail; following the first shock and then the first injection, a second defibrillation, and then, another medication injection may be tried again; amiodarone was removed from the protocol for asystole treatment because of relative lack of effectiveness).

The goal is avoidance of infarction, heart failure, and/or lethal arrhythmias (ventricular tachycardia, ventricular fibrillation, asystole, or pulseless electrical activity), and so ultimately to restore normal cardiac sinus rhythm. In any case, even if the cardiac event resolves quickly with few complications and emergency transport does not ensue, an urgent visit to the hospital or to one's physician or cardiologist is needed to narrow down the potential cause and ensure that, through testing, the heart and blood flow were not significantly and/or permanently impaired. Such incidents must never be downplayed or ignored, because the outcome of future episodes may not be so fortunate.

Materials and methods

The sample consisted of the University of Sarajevo students. The sample included students examined during regular and systematic examinations, and especially all the students who enroll and attend the Faculty of Physical Education the Department at the Institute for Public Health of students at the University of Sarajevo in the period 2007 - 2010. Personal history of student population age between 18-26 years involves assessing cardiac status of patients through all aspects of sports activities: type of sports activity, the beginning of training, duration and type of physical training loads, sports injuries, sudden cessation of training, etc. A very important stress is put on data on previous illnesses and family history.

Research methods:

a) Personal history data for assessment of cardiac status of the patient and family history of student at the University of Sarajevo;

b) Auscultatory data on cardiac rhythm and its regularity, intensity and quality of heart sounds, systolic and diastolic sounds, heart noises of the University of Sarajevo students;

c) ECG in the diagnosis of athletes' heart in students of the University of Sarajevo;

d) 24 h Holter-monitoring in the diagnosis of heart rhythm disorders of the athletes' heart in students at the University of Sarajevo;

e) Echocardiography and transesophageal finding in the diagnosis of athletes' heart in students at the University of Sarajevo.

Table	2.	gender	distribution	of	Athletes'	heart
syndro	эте	2				

Condor	Number of	Percentage	
Genuer	patients with AHS	%	
Male	16	41	
Female	2	2,86	
Σ	18	16,51	

Results

No significant difference was observed regarding gender distribution of athletes' heart syndrom in total sample (Table 2). Majority of examined patients is not committed to any type od sports or physical workout, with highly significant difference on level p<0,0000 for total sample with t=4,17 and t=8,45. and for female participants with t=7,09 and t=6,79. For male participants that difference is slightly less significant on level p<0005 with t=3,36 and t=3,74.

Gender	Туре	Number of participants	Percentage %
	Does not train	29	74
Male	Trains	7	18
	Trains occasionally	3	8
	Does not train	62	89
Female	Trains	3	4
	Trains occasionally	5	7
Σ	Does not train	91	84
	Trains	10	10
	Trains occasionally	8	6

Table 3. Display of participants sports activities

Type of disorder	Pq interval (shortened)		Qt Interva (Prolonged		l Nodal) Rhytms		Torsade De Pointes		Dilated cardiomyopathy		Hypertrophic cardiomyopathy		A-V Block			
	No	%	No	%	No	%	No	%	No	%		No	%	ó 0	No	%
AHS	1	0,92	7	6,42	3	2,75	1	0,92	6	5,50)	2	1,8	33	9	8,26
Heart rates, RR intervals					Average N		ax Min		Max	Max time		Min time		me		
HR [1/min]				68	2	13	30 38		25.8	25.8.2008 11:58		8 26.8		.2008 06:36		
HR equivalent intervals [ms]				88	<u>2</u> 9	20	90 430		26.8	26.8.2008.01.5		9 25 8 2008		2008	۲11· 4 1	
<u>BR</u> unfiltered [ms]			86	<u> 9</u>	53	50	0 200		25.8.2008 16:5		8	25.8.2008		13:48		
Lacny / brady / pause Number of events Longest event																
Tachycardia				42	429				25.8.2008 12:17			7	2 min 2 s			
Pause (2.5 s)				4	2				25.8	25.8.2008 16:		<u>9 29 min 5</u> 58 5,4 s		<u>n 5 s</u>		
ST elevation				10	107				25.8.2008.20.4			1	0.49 m)/ 2.0 mi			min
QRS statistics															11111	
Total Ma			Max/h	1		uratio	Lon	gest	jest HR [1/min] Du		Highest ration		st ra	ite 1/mii	1	
Veş		4000		101			uraut	211	1115 [1					LIIX [4
Couplet	Couplet 67 23			181												
Triplet 15 7			7													
Bigeminy	igeminy 21			12			3 s		128	128 0		S 1		195		
V rhym si	Trigeminy 133 V rihym sus 9			34			22 s		53	53 2		<u>s 1</u>		148		
RBBB		74														
SVES		176		40												
SV-tachycardia 2 1			1		00	00:00:05		94	94 00		:00:01 14		140)		
HRV tach	ogram															
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Display of segments from 24 h Holter-monitoring report of students tested for athletes' heart syndrome: Table of recorded heart rhythm disorders, HRV tachogram, ST chart, ST statistics, details of ECG-bigeminy, trigeminy, ventricular rhythm, ST elevation, bradycardia.

Display of segments from 24 h Holter-monitoring report of students tested for athletes' heart syndrome: Table of recorded heart rhythm disorders, HRV tachogram, ST chart, ST statistics, details of ECG-bigeminy, trigeminy, ventricular rhythm, ST elevation, bradycardia.

Discussion

Athletes' heart was found in: 16 boys (41%), 2 girls (2.86%) total of 18 patients (16.51%). Athletes' heart shows a series of functional and structural particularities. Specific electrocardiographs image

is usually in conjunction with morphological and functional signs of "athlete heart syndrome", such as increased heart volume, cost-effective functioning of the heart, and the ability to withstand high physical endurance. Athletes' heart is an expression of functional adaptation to the physical efforts. Frequency of electrocardiographic features is the greatest in athletes dealing with endurance sports and increases with the length of sports experience. Changes in the electrocardiogram of athletes' heart can be divided into several groups:

- 1) Changes related to "shift" of sympathicparasympathetic balance;
- 2) Changes attributable to myocardial hypertrophy;
- Changes which are difficult to distinguish from pathological conditions, repolarization disorder of chambers.

In the athlete often we see a turn to the right electrical axis. More often turn to the right axis is seen in young athletes of asthenic constitution. Sinus bradycardia at rest is the most common finding in the electrocardiogram in athletes-95%. It is believed that this is basic result of sinus node rate and vagal dominance effects, with reduced influence of the sympathetic regulation of heart rate.

Sinus bradycardia is accompanied by high indices of functional ability of the heart, and during exertion is replaced by tachycardia (86%). Sinus arrhythmia at rest is also a common finding in trained athletes-Tachyarrhythmia extrasystolica ventric.et supraventricularis in (10.09%) of the surveyed students. It is a consequence of changed sympathetic - parasympathetic balance in the regulation of heart rate. They can be caused by physiological or pathological causes. Regarding differential diagnosis an important place has the electrocardiograph record during endurance. If the extrasystole are unifocal, rare, disappearing during the endurance, and are not accompanied by clinical and laboratory abnormalities, probably it is the functional extrasystole.

If they are multifocal, and there is bigeminy, trigeminy, parasistolia, the phenomenon of R / T, or is transferred to other rhythm disturbances, along with clinical and laboratory abnormalities, decreased functional capacity, we consider them pathological findings.

Atrioventricular block of I grade is found in (8:26%) of students and is more common in well-trained athletes. It is attributed to the dominant role of the parasympathetic nervous system in a trained person, atrioventricular block grade II, Wenckebach type (Mobitz I) finding is more rare finding and is present in about (0.52%) of students. It is attributed to the shift of sympatho-parasympathetic balance. Since it may be the result of organic findings ergometric testing is required. Incomplete right bundle branch block is a common finding in athletes. Incomplete right bundle branch block is present in (14.68%) of students, its prevalence is higher among young elite athletes. It presents reflections of the hypertrophy of right ventricle. However such findings may be a sign of the existence of a dominant-atrium septal defect, and other arterial and ischemic diseases. A special doubt is raised by the incomplete transition to a complete right bundle branch block during the endurance test.

If QRS complex duration is normal, up to 0.10 seconds and there is a picture rR ', it is a normal finding that Reindel called physiological right bundle branch block (Many athletes have shown changes in repolarization, ventricular findings in the electrocardiogram, the ST elevation in (1.10%)of investigated students, as a characteristic of early repolarization, high-amplitude T-juvenile wave in the V2-V4 precordial leads is often in well trained athletes. T-wave inversions in precordial III lead are present in the extremities (46% - 89%) in the electrocardiogram of athletes. Even though such findings may be associated with mitral valvule prolapse and asymmetric hypertrophy of intraventricular septum (the extended PQ-int., the lack of septal Q-wave) and further cardiac diagnostic should be carried. The signs of left ventricular hypertrophy are often seen, and the voltage criteria are met by 1.83% well-trained athletes, students, and dilated cardiomyopathy in (5.50%) is present. Nodal rhythm (2.75%) and prolonged QT interval are present in (6:42%) of students.

In cross-sectional analysis, a spectrum of abnormal ECG patterns is present in $\approx 40\%$ of trained athletes, occurring 2-fold more commonly in men than women, and particularly in those participating in endurance sports Therefore, most athletes have ECGs that are within normal limits or that show only minimal alterations. However, the frequency with which these ECG patterns occur is highly dependent on the type, intensity, level of training, and precise criteria used to define normality .The most commonly reported alterations are early repolarization patterns, increased QRS voltages, diffuse T-wave inversion, and deep Q waves. Distinctly abnormal and bizarre ECGs, intuitively suggestive of cardiac disease, are encountered in an important minority of elite athletes ($\approx 15\%$).

The vast majority of such ECGs represent only extreme manifestations of physiological athlete's heart. Because of the heightened vagal tone that accompanies physical conditioning, trained athletes are known to commonly incur innocent arrhythmias and conduction alterations, such as sinus bradyarrhythmia, junctional rhythm, and first-degree or Wenckebach AV block (Mobitz type I). However, the application of ambulatory (Holter) ECG monitoring to trained athletes unexpectedly documented substantial ectopy with frequent premature beats and complex ventricular tachyarrhythmia (including couplets and bursts of non-sustained ventricular tachycardia) in many such individuals.

These findings suggest that a variety of arrhythmias are part of the athlete's heart spectrum .Indeed, such rhythm disturbances have not been associated with adverse clinical events and are usually abolished or substantially reduced after relatively brief periods of deconditioning (as well as during physical training sessions and exercise testing). Even in athletes with heart disease, resolution of ventricular tachyarrhythmia with deconditioning is common and may represent a potential mechanism by which sudden death risk is reduced by withdrawal of these individuals from training and competition,49,50 in accord with consensus panel recommendations. Ventricular arrhythmias in trained athletes. Top, Frequency of premature ventricular beats (PVBs), ventricular couplets, and bursts of non-sustained ventricular tachycardia (NSVT) recorded during a 24-hour (Holter) ECG at peak training and after a deconditioning period.

Heart related sudden death in young people

Heart related sudden death occurring in young people can be prevented. If you're at high risk of

sudden cardiac death, your doctor will usually suggest that you avoid competitive sports. Depending on your underlying condition, medical or surgical treatments may be recommended to reduce your risk of sudden death. Another option for some, such as those with hypertrophic cardiomyopathy, is an implantable cardioverter-defibrillator (ICD). This is a pager-sized device implanted in your chest like a pacemaker. An ICD continuously monitors your heartbeat. If a life-threatening arrhythmia occurs, the ICD delivers electrical shocks to restore a normal heart rhythm.

Screening for risk factors leading to cardiac related sudden death

There's debate in the medical community about screening young athletes to attempt to identify those at high risk of sudden death. Some countries, such as Italy and Japan, screen young people with the use of an electrocardiogram (ECG or EKG), which records the electrical signals present in the heart. However, this type of screening sometimes leads to false-positive results - indications that an abnormality or disease is present when in fact it is not — which can cause unnecessary worry and additional tests. It's not clear that sudden cardiac death can be prevented by the types of routine physical exams that are often necessary to be cleared to play competitive sports. There are some things you can do if you're worried about your risk factors. For example, if someone in your family dies young, it's important that an autopsy be done to determine the cause of death. If the autopsy shows that a heart condition caused the death, screening of family members may be necessary. For example, if the autopsy showed hypertrophic cardiomyopathy, it's recommended that all firstdegree relatives of the deceased should be checked for this condition, including parents, siblings and children. Repeat screening of family members is recommended over time, even if the first evaluation of their heart was normal.

Management of existing cardiac conditions

If you're at risk of sudden cardiac death, talk to your doctor about your physical activity level. Whether you can participate in exercise or sports depends on your condition. For some disorders, such as hypertrophic cardiomyopathy, it's often recommended that you avoid most competitive sports, but this does not mean that you will need to avoid exercise altogether. Talk to your doctor about what sort of activities and behaviors you should avoid. The recent sudden death of high school quarterback Reggie Garrett of West-Orange Stark High School, shocked and saddened his friends, family and the community. One minute the fit star quarterback was making a touchdown.

The next he collapsed and died. No one saw it coming. Each start of the new school year, headlines bear the names of a handful of young, seemingly healthy athletes who die suddenly on the basketball court, the football field or the track. The word is still out on what exactly caused Garrett to die. But when an otherwise superbly-conditioned teenager dies suddenly, a condition called sudden cardiac death is often to blame, say experts. Sudden cardiac death occurs when the heart stops abruptly. In teenagers and young adults, structural heart abnormalities or heart rhythm disturbances are a common cause of sudden death.

The condition claims the lives of a few athletes each fall (about 1 in 100,000 students a year), usually in the beginning of the sports season. Although physical exertion in the south Texas heat can create opportunity for other complications, it rarely is the underlying cause of sudden cardiac death. Newspapers splash headlines and communities are devastated because it is such an unexpected death in someone who was always thought to be healthy, an athlete. Typically the athletes have had symptoms as a warning, but they may have ignored it or assumed that they had just overdone it, over-exerted themselves or become too dehydrated. An inherited heart defect called hypertrophic cardiomyopathy is a common cause of sudden cardiac death. In persons with hypertrophic cardiomyopathy, the heart muscle-usually the left ventricle-is abnormally thickened.

The malformed ventricle can block the blood flow and cause abnormal heart rhythms, called arrhythmias. Some types of arrhythmias (not all) can lead to sudden death. In athletic teens with normal hearts, the heart muscle, like any other well-worked muscle, naturally thickens and enlarges from strenuous exercise so that it can pump more efficiently. Not to be confused with hypertrophic cardiomyopathy, this condition, called, "athlete's heart," is not a cause of sudden death. Marfan syndrome is another inherited disorder that increases risk for sudden death. This connective tissue disorder affects the skeleton, lungs, eyes, heart and blood vessels, and can cause aortic dilations, which can rupture suddenly when the heart is under stress. People with the syndrome are tall and have unusually long limbs, traits that are often desirable in sports like basketball. However, Marfan teens should avoid all contact sports. Coronary heart disease, congenital anomalies of the coronary arteries, some rare arrhythmias, aortic aneurysm dissection, myocarditis, long QT syndrome, Kawasaki's disease, and hard blows to the chest are also risk factors for sudden death. Fainting, chest pain, difficulty breathing and dizziness with exercise can all be warning signs of heart problems and warrant attention.

Conclusion

Athletes' heart was documented in 16 boys (41%), 2 girls (2.86%): total of 18 patients (16.51%). Following heart rhythm disturbances were recorded in athletes heart: bradycardia (95%), tachycardia (86 %); tachyarrhythmia extrasystolica ventricular et sup. in 11 patients (10.09%), atrial fibrillation in 2 boys (1.83%), RBBB in 16 patients with athletes' heart (14.68%), ST elevation was recorded in 23 patients, or (21.10%), out of that in 16 boys or (41.02%) and 7 girls, or (10.00%), ST depression was recorded in 21 patients, or (19.26%) out of which 6 boys or (15.38%) and 15 girls, or (21.42%), PQ interval was shortened in 1 patient, or (0.92%), prolonged QT interval in 7 patients, or 6. 42%, nodal rhythm and in 3 patients (2.75%) Torsade de pointes in a patient or 0.92%, AV block in 9 patients, or (8:26%). Dilated cardiomyopathy was recorded in 6 patients, or (5.50%); hypertrophic cardiomyopathy in 2 patients, or (1.83%).

Young competitive athletes are widely regarded as a special subgroup of healthy individuals with a unique lifestyle who are seemingly invulnerable and often capable of extraordinary physical achievement. For more than 100 years, there has been considerable interest in the effects of intense athletic conditioning on the cardiovascular system. The advent of echocardiography more than 30 years ago provided a noninvasive quantitative assessment of cardiac remodeling associated with systematic training, and consequently, a vast body of literature has been assembled that is focused on the constellation of alterations known as "athlete's heart." Athlete's heart is generally regarded as a benign increase in cardiac mass, with specific circulatory and cardiac morphological alterations, that represents a physiological adaptation to systematic training. However, the clinical profile of athlete's heart has expanded considerably over the last several years as a result of greater accessibility to large populations of trained athletes studied systematically with echocardiography, ECG, cardiac magnetic resonance, and ambulatory Holter ECG monitoring. As a consequence, there is increasing recognition of the impact that prolonged conditioning has on cardiac remodeling, which may eventually mimic certain pathological conditions with the potential for sudden death or disease progression. Over the last several years, sudden deaths of trained athletes, usually associated with exercise, have become highly visible events fueled by news media reports and with substantial impact on both the physician and lay communities.

Interest in these tragic events has accelerated owing to their increased recognition; awareness that underlying, clinically identifiable cardiovascular diseases are often responsible; and the availability of treatments to prevent sudden death for high-risk athlete-patients. In the present review, we offer a comprehensive assessment of many issues that target the interrelation of intense physical exertion with cardiac structure and function, as well as the rare, potentially adverse consequences of sports. If students answer yes to any of the questions, they may have an underlying heart problem that necessitates additional diagnostic testing by a pediatric cardiologist, such as an EKG and echocardiogram. Most of the time, the tests are only a precaution and the results reveal nothing wrong. But in some cases, these tests uncover dangerous heart abnormalities that a normal physical exam may miss-saving lives in the process. That's reason enough to make them a routine part of sports screenings for young athletes. We should add ECG and ECHO to the usual screening for all athletes (as well as non-athletes). Screening

by history and physical examination alone are not sufficient to detect many cardiovascular abnormalities in young athletes associated with sudden cardiac death.

From the above stated we come to the conclusion of the necessity of cardiac evaluation prior to the active involvement of youth in sports-enrolment to the Faculty of Physical Education, sports clubs, training, and regular cardiac monitoring during active sports-every 6 months electrocardiogram and ergometry of the heart and heart ultrasound once a year.

References

- 1. Maron BJ, Zipes DP. 36th Bethesda Conference: eligibility recommendations for competitive athletes with cardiovascular abnormalities. J Am Coll Cardiol. 2005; 45: 1312–1375.
- Pelliccia A, Maron BJ, DiPaolo FM, Biffi A, Quattrini FM, Pisicchio C, Roselli A, Caselli S, Culasso F. Prevalence and clinical significance of left atrial remodeling in competitive athletes. J Am Coll Cardiol. 2005; 46: 690–696.
- 3. Pelliccia A, Thompson PD. The genetics of left ventricular remodeling in competitive athletes. J Cardiovasc Med. 2006; 7: 267–270.
- 4. Neilan TG, Yoerger DM, Douglas PS, Marshall JE, Halpern EF, Lawlor D, Picard MH, Wood MJ. Persistent and reversible cardiac dysfunction among amateur marathon runners. Eur Heart J. 2006; 27: 1079–1084.
- 5. Maron BJ, Wentzel DC, Zenovich AG, Estes NAM III, Link MS. Death in a young athlete due to commotio cordis despite prompt external defibrillation. Heart Rhythm. 2005; 2: 991–993.
- 6. Sport category is an important determinant of cardiac adaptation: an MRI study Br. J. Sports. Med.. 2012;0:bjsports-2011-090520v1-bj-sports-2011-090520,
- 7. 2011 ACCF/AHA Guideline for the Diagnosis and Treatment of Hypertrophic Cardiomyopathy: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the American Association for Thoracic Surgery, American Society of Echocardiography, American Society of Nuclear Cardiology, Heart Failure Society of Ame-

rica, Heart Rhythm Society, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons J Am Coll Cardiol. 2011;58:e212e260,

- 8. 2011 ACCF/AHA Guideline for the Diagnosis and Treatment of Hypertrophic Cardiomyopathy: Executive Summary: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Developed in Collaboration With the American Association for Thoracic Surgery, American Society of Echocardiography, American Society of Nuclear Cardiology, Heart Failure Society of America, Heart Rhythm Society, Society for Cardiovascular Angiography and Interventions, and Society of Thoracic Surgeons J Am Coll Cardiol. 2011;58:2703-2738,
- 9. 2011 ACCF/AHA Guideline for the Diagnosis and Treatment of Hypertrophic Cardiomyopathy: Executive Summary: A Report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines Circulation. 2011;124:2761-2796,
- 10. Exercise-induced arrhythmogenic right ventricular cardiomyopathy: fact or fallacy? Eur Heart J. 2011;0:ehr436v1-ehr436,
- 11. 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy: Executive summary: A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines J. Thorac. Cardiovasc. Surg.. 2011;142:1303-1338,
- 2011 ACCF/AHA guideline for the diagnosis and treatment of hypertrophic cardiomyopathy: A report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines J. Thorac. Cardiovasc. Surg.. 2011;142:e153-e203,
- 13. Left ventricular systolic performance is improved in elite athletes Eur Heart J Cardiovasc Imaging. 2011;12:514-519,
- 14. The Athlete's Heart vs. the Failing Heart: Can Signaling Explain the Two Distinct Outcomes? Physiology. 2011;26:97-105,
- 15. Ventricular Remodeling: Fundamental to the Progression (and Regression) of Heart Failure J Am Coll Cardiol. 2011;57:1477-1479,
- 16. Relationship of ventricular and atrial dilatation to valvular function in endurance athletes Br. J. Sports. Med.. 2011;45:178-184,

- 17. Sudden cardiac death: clinical evaluation of paediatric family members Europace. 2011;13:421-426,
- 18. Head-to-head comparison between echocardiography and cardiac MRI in the evaluation of the athlete's heart Br. J. Sports. Med.. 2011;0:bjsm.2010.077669v1-bjsports77669,
- 19. Cardiac Arrhythmogenic Remodeling in a Rat Model of Long-Term Intensive Exercise Training Circulation. 2011;123:13-22,
- 20. Diagnosis and management of elite young athletes undergoing arrhythmia intervention Arch. Dis. Child. 2011;96:21-24,
- 21. De novo desmin-mutation N116S is associated with arrhythmogenic right ventricular cardiomyopathy Hum Mol Genet. 2010;19:4595-4607,
- 22. Echocardiographic deformation imaging reveals preserved regional systolic function in endurance athletes with left ventricular hypertrophy Br. J. Sports. Med.. 2010;44:872-878,
- 23. Review of the management of sudden cardiac arrest on the football field Br. J. Sports. Med.. 2010;44:540-545,
- 24. Long-Term Clinical Consequences of Intense, Uninterrupted Endurance Training in Olympic Athletes J Am Coll Cardiol. 2010;55:1619-1625,
- 25. Cardiac MRI reference values for athletes and nonathletes corrected for body surface area, training hours/week and sex European Journal of Cardiovascular Prevention & Rehabilitation. 2010;17:198-203,
- 26. Athletes at Risk for Sudden Cardiac Death The Journal of School Nursing. 2010;26:18-25,
- 27. Recommendations for interpretation of 12-lead electrocardiogram in the athlete Eur Heart J. 2010;31:243-259,
- 28. activity and physiological cardiac remodelling in a community setting: the Multi-Ethnic Study of Atherosclerosis (MESA) Heart. 2010;96:42-48,
- 29. Risk factors for exercise-related acute cardiac events. A case-control study Br. J. Sports. Med.. 2009;43:722-725,
- 30. Distinguishing hypertrophic cardiomyopathy from athlete's heart physiological remodelling: clinical significance, diagnostic strategies and implications for preparticipation screening Br. J. Sports. Med.. 2009;43:649-656,

- 31. Advances in cardiac imaging: the role of magnetic resonance imaging and computed tomography in identifying athletes at risk Br. J. Sports. Med.. 2009;43:677-684,
- 32. Commotio Cordis and the Epidemiology of Sudden Death in Competitive Lacrosse Pediatrics. 2009;124:966-971,
- *33.* 12-lead ECG in the athlete: physiological versus pathological abnormalities Br. J. Sports. Med.. 2009;43:669-676,
- 34. Echocardiographic tissue deformation imaging of right ventricular systolic function in endurance athletes Eur Heart J. 2009;30:969-977,
- 35. Sudden Deaths in Young Competitive Athletes: Analysis of 1866 Deaths in the United States, 1980-2006 Circulation. 2009;119:1085-1092,
- 36. CHAPTER 32 Sports and Heart Disease ESC Textbook of Cardiovascular Medicine. 2009;2:med-9780199566990-chapter;
- 37. Athlete Screening for Occult Cardiac Disease: No Risk, No Fun? J Am Coll Cardiol. 2008;51:1040-1041,
- Lohr, John. "Athletic Heart Syndrome." Gale Encyclopedia of Medicine, 3rd ed.. 2006. Encyclopedia.com. 6 Feb. 2012 < http://www.encyclopedia. com>.
- *39. Maron BJ, et al. Sudden deaths in young competitive athletes: Analysis of 1866 deaths in the United States, 1980-2006. Circulation. 2009;119:1085.*
- 40. Sealy DP, et al. Vital signs and demographics in the preparticipation sports exam: Do they help us find the elusive athlete at risk for sudden cardiac death? Current Sports Medicine Reports. 2010;9:338.

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Neurotoxic effects of Ropivacaine

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Abstract

Background: Ropivacaine is one of the newest long-acting local anesthetics. Local anesthetics used in clinical concentrations for perineural application and for the purpose of nerve blockade are not neurotoxic. However, when applied intra neurally, local anesthetics can exhibit neurotoxic effects. The purpose of this study is to determine neurotoxic effects of Ropivacaine.

Material and methods: Fifty Wistar rats were used in the study. After general anesthesia was administered to the animal sciatic nerves were exposed bilaterally. Total of one hundred sciatic nerves (50 rats) was randomized to receive: Ropivacaine or 0.9% NaCl, either intra neurally or perineurally. Pressure data was acquired by using a manometer coupled to a computer and was analyzed using software package BioBench. After the animals were awakened neurologic examination was performed thought the following 7 days after the experiment. Thereafter the rats were sacrificed while sciatic nerves were extracted and histological examination was performed.

Results: Intra neural injections in most of cases resulted with high injection pressure (p=109-187 kPa), followed by obvious neurologic deficit and microscopic destruction of peripheral nerves. In contrast to that, low injection pressure (p<24.5 kPa), applied either in perineural or intra neural extra fascicular area, resulted with transitory neurologic deficit and without destruction of the nerve normal histological structure. Using Ropivacaine instead 0.9% NaCl as injection solution during intra neural intra fascicular injection the level of nerve injury was increased.

Conclusions: Ropivacaine applied in intra fascicular area is neurotoxic, similar like any other local anesthetics. Ropivacaine applied in perineural space is potent long lasting local anesthetics appropriate for intraoperative and postoperative regional anesthesia and analgesia. **Key words:** Ropivacaine neurotoxicity, intra neural injection, perineural injection, nerve bloc-kade.

Introduction

Regional anesthesia has gained great popularity in the last decade. But, like any other medical procedure peripheral nerve blocks carry a certain risk of nerve injury. Unwanted complication during nerve blockades may occur after mechanical, ischemic or chemical injury. Injection of different local anesthetics has been shown to cause damage to the peripheral nerves (1-6).

Because of Ropivacaine's commonly favorable toxicity profile, it is likely to be used in peripheral nerve block. Ropivacaine is one of the newest amide-type long-acting local anesthetic that has become commonly used throughout Europe and USA since 1997. Many experimental studies and clinical experience till now have been shown that Ropivacaine is effective local anesthetic with greatest margin of safety, which produces less motor blockade in respect with level of sensitive analgesia (7,8).

Under normal clinical circumstances, when used in clinical concentration and for perineural application for the purpose of nerve blockade, including local anesthethesia, Ropivacaine is not neurotoxic. However, when applied intra neurally, local anesthetics can exhibit neurotoxic effects.

That is why the purpose of this study is to determine neurotoxic effects of Ropivacaine.

Hypothesis

Our hypothesis is that intra neural intra fascicular application of Ropivacaine (as documented by increased injection pressure during application) will result in neurologic injury. Also, our hypothesis is that perineural application and intra neural extra fascicular application of Ropivacaine does not lead to neurologic injury.

Material and methods

The study was conducted in accordance with the Principles of Laboratory Animal Care and approved by the Laboratory Animal Care and Use Committee. Fifty Wistar rats of both sexes were used in the study. On the day of experiment, general anesthesia (intraperitoneal injection of Nembutal-sodium pentobarbital 50mg/kg) was administered. After that, using aseptic technique for survival surgery, both sciatic nerves was exposed underneath the gluteus muscle through an incision. Total of one hundred sciatic nerves (50 rats) was randomly divided in two groups: intra neural and perineural group which received 0.75% Ropivacaine or 0.9% NaCl. For perineural injections needles was placed within the epineurial tissue but outside the perineurium; for intra neural injections: needles was placed intra neurally inside the perineurium. Increased injection pressure was used to distinguish intra fascicular from extra fascicular intra neural injections. Microscopic guidance was used for precise needle placement; the needle was stabilized using specific instrument (Activational Systems Inc., Scientific Instrumentation, SAS-1451AP, Small Animal Stereotaxic Frame, USA). Assignment to specific injection groups was done using a method of sealed envelopes. All injections was performed using a standardized protocol consisting of a 12.7-mm, 27-gauge, long-bevel needle (Terumo Europe NV, Leuven, Belgium) inserted at an angle of 45°-60°. Injections was carried out using an automated infusion pump (PHD 2000, Harvard Apparatus, Holliston, MA) with injection speed of 5 ml/min. Pressure data was acquired using an in-line manometer (PG5000, PSI-Tronics Technologies Inc, Tulare, CA) coupled to a computer via an analog-digital conversion board (DAQ 6023, National Instruments, Austin, TX) and analyzed using a data analysis software



Figure 1. Automatic syringe charger, manometer, PC laptop

package (BioBench version 1.2, National Instruments, Austin, TX), (Figure 1).

After injection, the incision was closed and the animal was allowed to recover. Another observer blinded to the specifics of the injection technique and the pressure data, performed neurological examination at 2-hour intervals for the first day after the animals were awakened and daily for 7 days thereafter. Neurological examination has been conducted by Thalhammer's neurological examination (9), and included assessment for the proprioception, motor function and nociception by the following criteria:

- Proprioception was evaluated by testing postural reactions (tactile placement response the rat was kept in a normal resting posture, toes of one foot were flexed with their dorsal part placed onto the supporting surface and the ability to reposition the toes was evaluated). The functional deficit was graded as: 0 normal; 1 slightly impaired; 2 severely impaired; 3 absent.
- Motor function was evaluated by measuring the extensor postural thrust: the rat was held upright with the hind limb extended so that the body's weight was supported by the distal metatarsus and toes and the extensor postural thrust could be measured as the force applied to the digital balance, the force that resists contact of the platform balance by the heel. The reduction in the force, representing reduced extensor muscle tone, was considered as a deficit of motor function and expressed as a percentage of the control force.
- Nociception was evaluated by observing the withdrawal of the limb in response to a noxious stimulation as:
 - 4-Normal withdrawal reaction, rapid withdrawal of the paw, vocalization, bites the forceps;
 - 3-Slower withdrawal reaction, slower withdrawal of the pinched extremity, vocalization, no attempts to bite the forceps;
 - 2-Slow withdrawal reaction, no vocalization, no attempts to bite the forceps;
 - 1-Barely perceptible withdrawal, no vocalization, no attempts to bite the forceps;
 - 0- no withdrawal, no vocalization, no attempts to bite the forceps;
The block duration was defined as time which passes until the response returns to score 3 (75 % of normal).

On the 7th day following the experiment, the rats were sacrificed (with overdoses of sodium pentobarbital and potassium chloride) and a 2-cm-long specimen of sciatic nerve (containing the injection site and approximately 1.0 cm proximal and distal to the injection site) was excised from the nerve. The tissues was fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin for histological examination. Series of tissue slices was taken throughout the specimen length. Pathologist blinded to the study groups performed histological analyses using light microscopy.

Statistical analysis

A study sample size of 100 sciatic nerves (50 rats) were required for the 80% power and a 5% type I error rate for a two-tailed T-test designed to detect a 1.5 SD difference in peak injection pressure in two groups defined as perineural vs. intra neural injections. Rates of neurologic and histological injuries were compared between intra neural and perineural injection by using McNemar's test for paired proportions. Fisher's exact test was used to compare injury rates during the intra neural injection, based on injected solution (Ropivacaine vs. 0.9% NaCl). Statistical analysis was performed by using SPSS and a p value of <0.05 was considered to be significant.

Results

The results of acquired application pressures

All injections were characterized by increase of pressure in the beginning of application, resulting in maximum pressure, which was then followed by significantly lower pressure during the remaining part of the application. Even though all perineural injections resulted with the pressure ≤ 24.5 kPa, the majority of intra neural injections were with the injection pressure ≥ 109.8 kPa.

During intra neural applications the maximum pressure was 187.3 kPa, while the minimum pressure was 26.4 kPa, achieved in peak effect. Maximum pressure reached in all perineural applications was 24.5 kPa and minimum was 14.6 kPa, also achieved in peak effect (Figure 2.).



Figure 2. Intra neural and perineural injection application in rats

The average value of maximum pressure achieved in peak effect for intra neural injection was 138.1 \pm 30.9 kPa (mean value \pm standard deviation), in comparison to 16.9 \pm 1.9 kPa for perineural injection (p \leq 0.05). The difference between average values of intra and perineural injections (with 95% confidence interval) was statistically significant (t=3.14; DF=6; p=0.02). Only two intra neural injections resulted in lower injection pressures which are indicated as intra neural extra fascicular injections.

Results of neurological examination of experimental animals

After neurological exam, it has been found that all intra neural injections which were associated with high application pressure resulted with deficits which lasted more than 24 hours, and neurological deficits were evident also at the end of experiment, after 7 days, which clearly shows that intra neural intra fascicular injection caused the nerve damage. On the contrary, all injections associated with low injection pressure, whether they were intra neural or perineural didn't result with neurological sequels at the end of the experiment (p<0.05). Furthermore, in most cases neurological deficit has withdrawn within first 24 hours of experiment, (Figures 3,4,5).

Results of histopathological examination

After the injections, the nerves were found to be locally enlarged at the injection site. In 3 intra



Figure 3. Proprioception of hind limb after injection application of Ropivacaine



Figure 4. Motor function of hind limb after injection application of Ropivacaine



Figure 5. Nociception of hind limb after injection application of Ropivacaine

neural injections under the high pressure hyperemia of the epineurial vessels was noted.Histological examination revealed normal sciatic nerve structure in all low pressure injections (50 perineural and 2 intra neural extra fascicular). Perineurial and extra fascicular injections did not result in any significant nerve injury as assessed by light microscopy. By contrast, pathological changes which varied from occasional nerve fiber injury at the site of injection to severe axonal and myelin degeneration were observed following intra fascicular injections. In addition marked cellular infiltration, subperineurial edema and diffuse axonal swelling was apparent in most of intra fascicular injections. Pathological conditions in the periphery of the fascicle were more prominent than in central zone. With Ropivacaine injected intra fasciculary pathological findings were more marked, with evidence of wide-spread axonal and myelin degeneration of the entire fascicle. The high injection pressure group had a significantly greater rate of injury (100%) as compared with the low injection pressure group. Using Ropivacaine instead of 0.9% NaCl during intra fascicular injections the degree of nerve injury was increased (0%; Fisher exact test p=0.03).



Figure 6. Intra neural injection with 0.9% NaCl under high pressure (HE, X 40)

Epineurium is shown with hyper cellularity of the mononuclear inflammatory process type. Groups of adipocytes with hyperemic blood vessels are noted. Perineurium is shown as division of lamellas with its significant disintegration, while nerve fibers evidence of nerve injury.



Figure 7. Intra neural injection with 0.9% NaCl under high pressure (HE, X 100)

Diffuse damage of nerve fibers.



Elegend: Pe-perineurium Nj-nerve fibers Er- erythrocytes Figure 8. Intra neural injection with 0.9% NaCl under high pressure (HE, X 250)

Nerve fibers are disarranged in the space and of increased volume. Most of the axons of those fibers are dislocated and hyperacidophile. Advanced axolysis and myelin disintegration is noticed. Some of the erythrocytes are located extravasally.



Legend: Pe-perineurium Nf-nerve fibers Figure 9. Intra neural injection with Ropivacaine under high pressure (HE, X100)

Perineurium is shown as division of lamellas, with evidences of subperineural edema. Degenerative changes through entire fasciculus are noted.



Legend: Pe-perineurium Sc-Schwann's cells Figure 10. Intra neural injection with Ropivacaine under high pressure (HE, X 250)

Diffuse axonal swelling and an advanced axolysis up to degree of complete disintegration was apparent.



Legend: Nf-nerve fibers Figure 11. Intra neural injection with Ropivacaine under high pressure (HE, X 400)

Diffuse damage with evidence of axon degeneration. No normal axons are seen. Schwann's cells are enlarged with hyperchromatic nuclei.

Discussion

From the previous studies it is known that all local anesthetics are potentially neurotoxic if they have been used in higher concentrations than prescribed or if they act on nerve through prolonged time period (10). However, the previous experience shows that perineural application of local anesthetic significantly reduces neurotoxic potential, meaning that it carries very small risk of nerve damage. The reason for this is probably the fact that in normal circumstances applied amount of local anesthetic equalizes pressure with surrounding tissue. In that moment the diffusion into surrounding tissue occurs, the interstitial liquid rapidly dilutes local anesthetic and its concentration further decreases by system absorption. As in previous studies, in our study as well all perineural injections of local anesthetic (appropriate doses and concentrations) have not resulted with significant damage of nerve fibers.

In contrast to perineural injections, the intra neural injections of local anesthetic may result with nerve damage (11,12). Our results correspond with results from previous studies showing that intra neural injection increases the risk of nerve damage.

While some authors consider that for the emergence of nerve defect multi-factorial impact is needed (mechanical trauma and toxic effect of local anesthetic), others showed that the main cause of nerve injury during application of intra fascicular injection is mechanical trauma, depending on the kind and dose of applied solution and on the addition of epinephrine, we can find various types of nerve damages (13). In our study most of intra neural injections, independent to the kind of applied solution, also associated with high injection pressure (48 out of 50 injections) have resulted with persistent neurological deficit, which shows that mechanical insult caused by intra fascicular placed needle is critical in the occurrence of nerve injury. In other words, our results show that the place of application is crucial factor in determination the grade of nerve injury. With the intra fascicular injection of Ropivacaine instead 0.9% NaCl the level of nerve injury is increased.

Contrary to our and many others results Iohom and associates in 2005 applied intra neurally Ropivacaine into sciatic nerve of rat and concluded that intra neural injection of Ropivacaine has no noxious effect on nerve motor function (14). Unfortunately authors ignored that intra neural injection can be intra or extra fascicular, what would have great impact on final outcome. Also, authors analyzed only motor function, without examination of sensory function and without histological verification of those findings. Whitlock et al showed that Ropivacaine is associated with marked histological abnormality when injected intra fasciculary, while milder histological damage were seen when Ropivacaine was injected extra neuraly or extra fasciculary (15). The authors used finger pressure to distinguish intra fascicular and extra fascicular injections. Unfortunately, anesthesiologists often rely on subjective estimation of abnormal resistance to injection using finger pressure during the performance of peripheral nerve block, knowing that intra neural injection results with bigger resistance to needle. Hadzic and associates showed that the perception of the resistance can rather vary among the anesthesiologists, that this method is inconsistent and can be affected by different designs of needles (16).

Selander and Hadzic have demonstrated that intra neural injections into sciatic nerve of the dog

in most of cases were combined with high injection pressure, while perineural injections were associated with low application pressure (17,18). In our study 48 intra neural injections were combined with injection pressure higher than 109 kPa, while neither one perineural injection was resulted with pressure higher than 24.5 kPa. Even more important, intra neural high injection pressures in our study were also associated both with neurological deficit and histological evidence of injury to nerve fascicles.

Two low pressure intra neural injections did not result in neurological consequences because the needles were not lodged intra neurally but between the fascicles instead of intra fasciculary. Since peripheral nerves have natural protective mechanisms, like relative resistant membrane of perineurium, it is hard to assure intra fascicular lodged needle. In that case local anesthetic is deposited out of fascicle and such blockade lasts for hours after injection, but there is no histological evidence of nerve fibers damage.

In our study fascicular injury and neurological deficit were developed only after intra neural injection joined with high injection pressure. Study of Kapur and associated gave similar results (19). In fact, they used sciatic nerves of dogs and injected 20 intra neural injections in which only 8 resulted with intra fascicular lodged needle and were combined with high injection pressure. Remaining 12 intra neural injections were combined with lower injection pressure and there was no evidence of nerve fibers injury. Opposed to the mentioned study in our study number of intra neural extra fascicular injection was much lower (2 from 50). The reason is that sciatic nerves differ in rats and dogs. In rats nerve is composed mostly from 1 big and 1-2 small fascicles with little epineural tissue. It is not case with sciatic nerve of dogs, pigs, rabbit and humans, where nerve is mostly multifascicular or composed from more equal fascicles with extensive epineurium. This is the reason why in other species was more difficult lodging the needle intra fasciculary than in our case. Besides proven neurotoxic properties of Ropivacaine when injected intra fasciculary, feature which is characteristic for all other local anesthetics, Ropivacaine applied perineuraly is good choice for intraoperative and postoperative regional anesthesia and analgesia.

Conclusions

Ropivacaine applied in intra fascicular space is neurotoxic, similar like any other local anesthetics;

Ropivacaine applied in perineural area is potent long lasting local anesthetic appropriate for intraoperative and postoperative regional anesthesia and analgesia;

The main mechanism which lead to neurologic injury combined with peripheral nerve blockade is intra fascicular injection;

The prerequisite for avoiding intra neural intra fascicular application and consecutive damaging of peripheral nerve is differentiating nerve structures based on pressures registered by manometer.

References

- 1. Kalichman MW, Powell HC, Myers RR. Quantitative histologic analysis of local anesthetic-induced injury to rat sciatic nerve. J Pharmacol Exp Ther. 1989 Jul;250(1):406-13.
- 2. Powell HC, Kalichman MW, Garrett RS, Myers RR. Selective vulnerability of unmyelinated fiber Schwann cells in nerves exposed to local anesthetics. Lab Invest. 1988 Aug;59(2):271-80.
- 3. Hertl MC, Hagberg PK, Hunter DA, Mackinnon SE, Langer JC. Intrafascicular injection of ammonium sulfate and bupivacaine in peripheral nerves of neonatal and juvenile rats. Reg Anesth Pain Med. 1998 Mar-Apr; 23(2):152-8.
- 4. Mackinnon SE, Hudson AR, Bojanowski V, Hunter DA, Maraghi E. Peripheral nerve injection injury with purified bovine collagen--an experimental model in the rat. Ann Plast Surg. 1985 May;14(5):428-36.
- 5. Auroy Y, Benhamou D, Bargues L, Ecoffey C, Falissard B, Mercier FJ, Bouaziz H, Samii K. Major complications of regional anesthesia in France: The SOS Regional Anesthesia Hotline Service. Anesthesiology. 2002 Nov;97(5):1274-80.
- 6. Gentili F, Hudson AR, Hunter D, Kline DG. Nerve injection injury with local anesthetic agents: a light and electron microscopic, fluorescent microscopic, and horseradish peroxidase study. Neurosurgery. 1980 Mar;6(3):263-72.
- Kuthiala G, Chaudhary G. Ropivacaine: A review of its pharmacology and clinical use. Indian J Anaesth. 2011 Mar;55(2):104-10.

- 8. Fredrickson MJ, Smith KR, Wong AC. Importance of volume and concentration for ropivacaine interscalene block in preventing recovery room pain and minimizing motor block after shoulder surgery. Anesthesiology. 2010 Jun;112(6):1374-81.
- 9. Thalhammer JG, Vladimirova M, Bershadshy B, Strichartz GR. Neurologic evaluation of the rat during sciatic nerve block with lidocaine. Anesthesiology 1995;82:1013-25.
- 10. Chambers W.A. (1992). Peripheral nerve damage and regional anesthesia. Br.J.Anaesth. 1992; 69:429-430.
- Vučkovic I., Hadžić A., Dilberović F., Kulenović A., Mornjaković Z., Zulić I., Divanović K.A., Kapur E., Čosović E., Voljevica A. Detection of neurovascular structures using injection pressure in blockade of brachial plexus in rat, Bosn. J. Med. Sci.2005;5(3):79-85.
- 12. Selander D. Peripheral nerve injury caused by injection needles. Br J Anaesth. 1993 Aug;71(2):323-5.
- 13. Mornjaković Z, Dilberović F, Cosović E, Divanović KA, Zaciragić A, Kapur E, Vucković I. Histological changes of the sciatic nerve in dogs after intraneural application of lidocaine--relation to the established application pressure. Bosn J Basic Med Sci. 2005 Feb;5(1):8-13.
- 14. Iohom G, Lan GB, Diarra DP, Grignon Y, Kinirons BP, Girard F, Merle M, Granier G, Cahn V, Bouaziz H. Long-term evaluation of motor function following intraneural injection of ropivacaine using walking track analysis in rats. Br J Anaesth. 2005 Apr;94(4):524-9.
- 15. Whitlock EL, Brenner MJ, Fox IK, Moradzadeh A, Hunter DA, Mackinnon SE. Ropivacaine-induced peripheral nerve injection injury in the rodent model. Anesth Analg. 2010 Jul;111(1):214-20.
- Claudio R, Hadzic A, Shih H, Vloka JD, Castro J, Koscielniak-Nielsen Z, Thys DM, Santos AC. Injection pressures by anesthesiologists during simulated peripheral nerve block. Reg Anesth Pain Med. 2004 May-Jun; 29(3): 201-5.
- Selander D, Brattsand R, Lundborg G, Nordborg C, Olsson Y. Local anesthetics: importance of mode of application, concentration and adrenaline for the appearance of nerve lesions. An experimental study of axonal degeneration and barrier damage after intrafascicular injection or topical application of bupivacaine (Marcain). Acta Anaesthesiol Scand. 1979 Apr; 23(2):127-36.

- Hadžić A, Dilberović F, Shah S, Kulenović A, Kapur E, Začiragić A, Čosović E, Vučković I, Divanović KA, Mornjaković Z, Thys DM, Santos AC. Combination of intraneural injection and high injection pressure leads to fascicular injury and neurologic deficits in dogs. Reg Anesth Pain Med. 2004 Sep-Oct; 29(5):417-23.
- 19. Kapur E, Vuckovic I, Dilberovic F, Zaciragic A, Cosovic E, Divanovic KA, Mornjakovic Z, Babic M, Borgeat A, Thys DM, Hadzic A. Neurologic and histologic outcome after intraneural injections of lidocaine in canine sciatic nerves. Acta Anaesthesiol Scand. 2007 Jan; 51(1):101-7.

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References

- Sehane T, Takeno M, Sazaki N, Inaha G. Bohant's dianasa. N Engl J Mail 1999; 341: 1284–1291.
- Stonert SM, Leve TH, Beston CL, et al. A Prospective Analysis of Stress and Academic Performance in the first two years of Medical School. Med Educ 1999; 33(4): 243–50.

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