YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2016; February Vol. 73 (No. 2): p. 125–216.

YU ISSN 0042-8450 vol. 73, br. 2, 2016.

VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine

Časopis nastavlja tradiciju *Vojno-sanitetskog glasnika*, koji je izlazio od 1930. do 1941. godine

IZDAVAČ
Uprava za vojno zdravstvo MO Srbije

Adresa redakcije: Vojnomedicinska akademija, Institut za naučne informacije, Crnotravska 17, poštanski fah 33–55, 11 040 Beograd, Srbija. Informacije o pretplati: Tel.: +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

Radove objavljene u „Vojnosanitetskom pregledu“ indeksiraju: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, Index Medicus (Medline), Excerpta Medica (EMBASE), EBSCO, Biomedicina Serbica. Sadržaje objavljuju Giornale di Medicine Militare i Revista de Medicina Militara. Prikaze originalnih radova i izvoda iz sadržaja objavljuje International Review of the Armed Forces Medical Services.

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 1227423129552141 5. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € (u dinarskoj protivvrednosti na dan uplate) za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa Vojna štamparija, Beograd, Resavska 40 b.

YU ISSN 0042-8450 vol. 73 No. 2, 2016

VOJNOSANITETSKI PREGLED

The first issue of *Vojnosanitetski pregled* was published in September 1944
The Journal continues the tradition of *Vojno-sanitetski glasnik* which was published between 1930 and 1941

PUBLISHER
Military Health Department, Ministry of Defence, Belgrade, Serbia

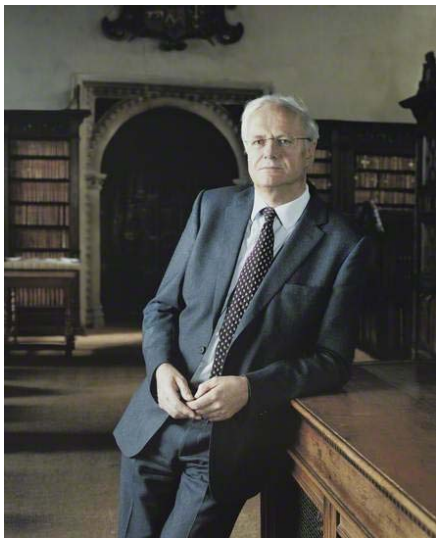
Editorial Office: Military Medical Academy, Institute for Scientific Information, Crnotravska 17, PO Box 33–55, 11 040 Belgrade, Serbia. E-mail: **vsp@vma.mod.gov.rs**

Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, Index Medicus (Medline), Excerpta Medica (EMBASE), EBSCO, Biomedicina Serbica. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.

The Journal is published monthly. Subscription: Giro Account No. 840-314849-70 Ministry of Defence – Total means of payment – VMA (for the Vojnosanitetski pregled), refer to number 12274231295521415. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40 b.

CONTENTS / SADRŽAJ



Christopher M. Dobson, FRS, FMedSci (born on October 8, 1949), a professor of Chemical and Structural Biology in the Department of Chemistry at the University of Cambridge, UK, is known worldwide for research dealing with protein folding and misfolding, and their links with neurodegenerative disorders such are Alzheimer's and Parkinson's diseases. The major goal of his research is to elucidate the general molecular principles that underlie these medical conditions and to generate a firm foundation for their rational and effective prevention and treatment. Prof. Dobson is the author or co-author of nearly 700 papers and review articles, including more than 30 in the journals Nature and Science, which have been cited over 50,000 times. In October last year, at the Workshop “Proteins and Beyond”, held at the Leiden University (The Netherlands), Prof. Dobson delivered a remarkable lecture on this topic (see p. 211-2).

Kristofer M. Dobson, FRS, FmedSci (rođen 8. oktobra 1949), profesor hemije i strukturne biologije na Univerzitetu Kembridž, Velika Britanija, poznat je širom sveta po istraživanjima u oblasti normalnog i patološkog savijanja proteina i njihove veza sa poremećajima kao što su Alchajmerova i Parkinsonova bolest. Glavni cilj ovih istraživanja je da se razjasne opšti molekularni principi koji leže u osnovi tih poremećaja i postave čvrste osnove za njihovu racionalnu i efikasnu prevenciju i lečenje. Profesor Dobson je autor ili koautor blizu 700 članaka, uključujući više od 30 objavljenih u časopisima *Nature* i *Science*, koji su citirani preko 50 000 puta. U oktobru prošle godine, na skupu „Budućnost proteina“ koji je održan na Univerzitetu u Lajdenu, Holandija, prof. Dobson je održao izvanredno predavanje na tu temu (vidi str. 211-2).

ORIGINAL ARTICLES

UDC: 616.314-003.4-053.2
DOI: 10.2298/VSP140828147P

Retrospective study of spontaneous bone regeneration after decompression of large odontogenic cystic lesions in children

Retrospetkivna studija spontane regeneracije kosti posle dekompresije velikih odontogenih cističnih lezija kod dece

Marko Pejović*, Jelena Stepić*, Aleksa Marković*, Miroslav Dragović*, Biljana Miličić†, Snježana Čolić*

***Clinic of Oral Surgery, †Department of Medical Statistics and Informatics, Faculty of Dental Medicine, University of Belgrade, Belgrade, Serbia**

Abstract

Background/Aim. Surgical treatment of odontogenic cysts in childhood could be accompanied by injury of important anatomical structures. Even though enucleation is considered to be preferable treatment of odontogenic cysts, the specificities of pediatric age favor more conservative surgical approach. The aim of this study was to assess the effectiveness of decompression as the uttermost treatment of odontogenic cysts in the pediatric age. **Methods.** This retrospective study included 22 patients, 7−16 years old, with a single jaw cystic lesion. The majority of these lesions were dentigerous cyst (14), and the rest belonged to keratocystic odontogenic tumor (KCOT) (8). All lesions were primarily treated with decompression; it was a final treatment (one-stage procedure) in 13 dentigerous cysts, and it was followed by enucleation (two-stage procedure) in one dentigerous cyst and all the KCOT. **Results.** A total of 13 (59.1%) dentigerous cysts were treated successfully only with decompression as one stage procedure, while the other 9 (40.9%) cysts required enucleation (1 dentigerous and 8 KCOT), after decompression ($p \leq 0.001$). **Conclusion.** Related to non-aggressive lesions, more conservative treatment approach, such as decompression as one-stage procedure, should be considered. On the other hand, KCOTs in children require a two-stage procedure for a successful treatment outcome.

Key words:
odontogenic cysts; oral surgical procedures; decompression, surgical; treatment outcome; pediatric dentistry.

Apstrakt

Uvod/Cilj. Hirurška terapija odontogenih cista u dečjem uzrastu može da bude udružena sa povredama važnih anatomskih struktura. Mada se enukleacija smatra terapijom izbora viličnih cista, specifičnosti dečjeg uzrasta nameću primenu konzervativnijeg hirurškog pristupa. Cilj istraživanja bio je da se proceni efikasnost dekompresije kao definitivnog terapijskog modaliteta kod odontogenih cista u dečjem uzrastu. **Metode.** Ova retrospektivna studija obuhvatila je 22 pacijenta, uzrasta 7−16 godina, sa solitarnim cističnim lezijama. Najčešće među njima su bile folikularne ciste (14), a ostale su bile keratocistični odontogeni tumori (KCOT). Sve cistične lezije prvo su tretirane dekompresijom; to je bio i jedini poduhvat (jednofazni postupak) kod 13 folikularnih cista, a potreba za sekundarnim zahvatom – enukleacijom, ukazala se posle dekompresije jedne folikularne ciste i svih KCOT (dvofazni postupak). **Rezultati.** Ukupno 13 (59,1%) cističnih lezija, od kojih su sve bile folikularne ciste, uspešno je podvrgnuto jednofaznom hirurškom postupku, dok je preostalih 9 (40,9%) cista (1 folikularna i 8 KCOT) zahtevalo naknadnu enukleaciju. **Zaključak.** Kod neagresivnih cističnih lezija preporučuje se konzervativniji hirurški pristup kao što je dekompresija (jednofazna procedura). Nasuprot tome, KCOT u dečjem uzrastu zahtevaju dvofazni hirurški postupak − dekompresiju praćenu enukleacijom.

Ključne reči:
ciste, odontogene; hirurgija, oralna, procedure; dekompresija, hirurška; lečenje, ishod; stomatologija, dečija.

Introduction

Although the frequency of odontogenic cysts is relatively low in children, three- dimensional growth of maxillofacial skeleton as well as odontogenesis of deciduous and permanent dentitions during the pediatric age might be associated with developmental jaw cysts formation [1, 2]. When occur, they exhibit rapid growth and large size, causing bone expansion,

Correspondence to: Marko Pejović, Clinic of Oral Surgery, Faculty of Dental Medicine, University of Belgrade, Rankeova 4, 11 000 Belgrade, Serbia. Phone: +381 63 219 925. E-mail: marko.implant@gmail.com

displacement of adjacent teeth, root deformation of developing permanent teeth or an intraoral buccal swelling [3]. Treatment of such lesions is solely surgical.

An aggressive management of large cysts in children can have an adverse effect on tooth development, eruption process, and growth of the involved jaw [4]. Therefore, less aggressive surgical treatment might be beneficial for pediatric patients due to anatomic specificities of the growing facial bones, existence of permanent tooth germs, faster bone repair process [5], and difficulties in cooperation. Decompression through an opening into the cystic cavity releases intramural pressure, favors formation of new bone, provides not only preservation of neurovascular and other anatomic structures but also minimal morbidity and low rate of surgical complications, including possible recurrence and decreased aggressiveness of the lesions [6, 7]. Therefore, a conservative treatment approach is especially interesting to be performed in patients of pediatric age.

In the literature there is not much information relating to conservative surgical treatment approach to odontogenic jaw cysts in childhood. The aim of this retrospective study was to evaluate the effectiveness of decompression as the uttermost treatment of large odontogenic jaw cysts in pediatric age.

Methods

A total of 22 young and healthy patients (15 males, 7 females) with a large odontogenic cystic lesion treated by decompression between the year 2002 and 2011 were included in this research. The mean age of the patients was 11.3 ± 2.9 (range 7.5−16) years. Referring to histopathological finding, there were 14 dentigerous cysts and 8 keratocystic odontogenic tumors (KCOT). The data were collected reviewing medical files and panoramic radiographs of patients under the age of sixteen, who were subjected to decompression of odontogenic cystic lesions > 2 cm in diameter. The patients with confirmed nevoid basal cell carcinoma syndrome, as well as patients with small, multiple or recurrent lesions, were excluded from the research.

Clinical data, such as patient age and gender, chief complaints, localization and size of the lesion, affected teeth, decompression time, definite surgery modality, complications, recurrences, follow-up period and the histopathological diagnosis were recorded. The size of the cyst and its relation to the teeth and adjacent structures were evaluated and compared on panoramic radiographs made before and after the decompression. The size of the cyst was recorded as maximal vertical diameter (in cm), maximal horizontal diameter (in cm) and their multiplication (in cm^2) named standard lesion area index (SLAI) [8].

All cystic lesions were treated with decompression performed by one experienced oral surgeon under local anesthesia (2% lidocaine with epinephrine 1:100,000). Decompression implied small bony window preparation for opening the cystic lesion cavity and biopsy sample collection at the same time. The opening was maintained either by polyethylene tube or iodoform gauze. A polyethylene tube was placed in the socket if deciduous tooth was extracted simultaneously or otherwise on the top of the alveolar ridge and fixed by suturing to surrounding mucosa. Whenever the tube was instable or the cyst was positioned buccally, the opening was maintained with iodoform gauze replaced after irrigation. All the remaining cavities were rinsed with sterile saline solution three times a week.

Control radiographic examinations were performed in a 4-month period. Control radiographs were used to evaluate the treatment success − the size of the remaining cavity and the position of the impacted teeth. Irrigation was performed until the remaining cavity had been shrunk completely or tooth erupted spontaneously. Just in cases where a part of the dentigerous cyst stayed trapped or when KCOT was diagnosed, enucleation was performed in the second act, when panoramic radiograph showed a significant reduction in the size of the remaining cavity without involving vital anatomical structures. In addition, Carnoy solution was always used in order to minimize the recurrence rate of KCOT.

One year after the end of the therapy an independent observer evaluated bone regeneration of the treated area.

This study was approved by the local institutional board. The principles outlined in the Declaration of Helsinki were fllowed.

Statistical analysis

For comparison between different histopathological findings Student's *t*-test, the χ^2 test, Fisher's test and Mann-Whitney *U* test were used. Univariate and multivariate binary logistic regression analysis were used to evaluate the relationship between different odontogenic cystic lesion and potential determinants. The results were expressed as the odd ratios (OR) and their 95% confidence intervals (CI). Statistical analyses were performed using SPSS (SPSS version 19.0 Inc., IBM Chicago). A statistical significance was defined as $p < 0.05$.

Results

According to localization of lesions, there was no significant difference between jaws (Table 1). Nevertheless, it was found that 6 out of 14 dentigerous cysts were located in the maxilla (3 in the frontal region, 1 in the posterior region and 2 affected both of frontal and posterior region). The remaining 8 were in the mandible (7 in the posterior region and 1 extending to the frontal region). KCOTs were localized as follows: 3 were in the maxilla (1 in the frontal region, 1 in the posterior and 1 spreading from anterior to posterior region), and 5 in the mandible, predominately in the angle/ramus, except 1, which was found in the region of the lower canine.

Although the average size of KCOTs was higher than the average size of the dentigerous cysts, there was no significant difference as between jaws (Table 1). There was a significant difference between the mean age of the patients with KCOTs and those with dentigerous cysts (Table 1).

A total of 15 (68.2%) cysts were discovered accidentally while 7 (31.8%) cysts (5 dentigerous and 2 KCOTs) had some clinical symptoms (swelling, oozing cystic fluid, etc.), but without anyone being predominant. A

Table 1

Demographic and clinical characteristics of patients regarding histopathological (HP) findings

Patient and lesion characteristics	HP findings		*p*
	Dentigerous cyst	KCOT	
Gender, n (%)			
male	9 (64.3)	6 (75.0)	0.490
female	5 (35.7)	2 (25.0)	
Age (years), $\bar{x}$ ± SD; med; min−max	9.5 ± 1.5; 9.0; 7.5−13	14.3 ± 2.2; 15.75; 11−16	≤ 0.001*
Size (cm^2), $\bar{x}$ ± SD; med; min−max	4.2 ± 1.5; 4.2; 2.0−7.1	6.6 ± 4.07; 6.06; 2.6−15	0.143[a]
Jaw, n (%)			
maxilla	6 (42.9)	3 (37.5)	0.584[c]
mandible	8 (57.1)	5 (62.5)	
Therapeutic method, n (%)			
decompression	13 (92.9)	0 (0)	≤ 0.001*[c]
decompression + enucleation	1 (7.1)	8 (100.0)	
Decompression time (months), $\bar{x}$ ± SD; med; min−max	6.9 ± 1.7; 6.0; 6−12	10.3 ± 3.2; 10.5; 6−16	0.006*[b]

[a]*t*-test; [b]Mann-Whitney *U*-test; [c]Fisher's test; *statistically significant; SD – standard deviation; med − median; KCOT – keratocystic odontogenic tumor.

total of 13 cysts were treated successfully only with the one-stage procedure while the other 9 required enucleation (Table 1). The mean duration of decompression was 8.2 ± 2.9 months for all cystic lesions, regardless of histopathological findings. We found that the mean duration of decompression was significantly shorter in those patients who had been treated only by decompression (7.00 ± 0.5 months, $p = 0.022$). The mean duration of decompression preceding enucleation was 9.9 ± 1.1 months, which was significantly different. Minimal duration of decompression was 6 months and maximal one 16 months.

It was discovered that 75% of KCOTs had impacted tooth in the cystic lumen (Figures 1a and b). It was the third molar that was impacted most often (83.33%) and all of them were extracted during enucleation (Figure 1c); 10 (77.77%) dentigerous cysts were associated with premolars − 8 (66.66%) mandibular and 2 (11.11%) maxillary. A total of 4 (22.22%) maxillary dentigerous cysts were associated with the canine. Just in one (7.14%) case the affected tooth was extracted, while in 13 (92.86%) patients the affected teeth were saved; of these, 12 (85.72%) teeth erupted spontaneously and 1 (7.14%) needed orthodontic traction (Figure 2a−c). Figure 3 shows the rough surface of active bone deposition favored by decompression in one patient.

Fig. 1 − Preoperative panoramic radiograph shows a large radiolucent cystic lesion with the impacted third molar located at the base of the mandible [keratocystic odontogenic tumor (KCOT)]; b) Six months after decompression, the impacted third molar was moved to a more suitable position, making extraction much easier. Ongoing bone deposition decreased a defect, making enucleation less traumatic; c) Three years after enucleation, panoramic radiograph shows a complete healing of the former bone defect, without any suspicious sign of a recurrence.

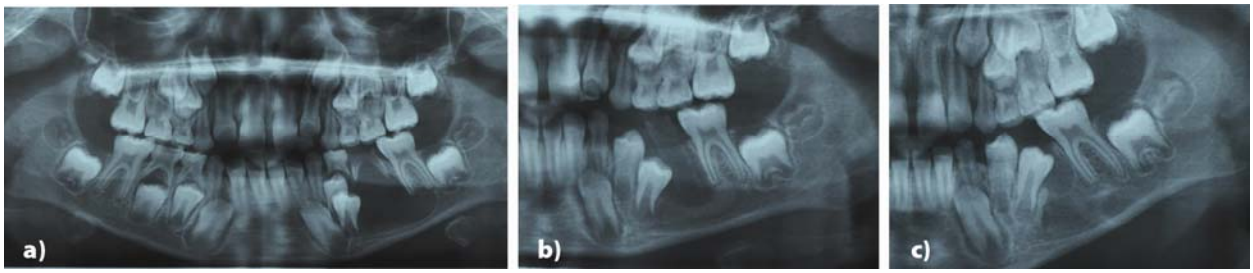


Fig. 2 − a) Panoramic radiograph shows a dentigerous cyst involving permanent premolars germs; b) After four months of decompression, radiograph view shows a polyethylene tube in the defect and newly formed bone layer with permanent premolars' germs eruption through cystic cavity; c) After nine months of decompression, a panoramic radiograph shows complete resolution of the lesion and moving the involved teeth into physiologically determined position within dental arches.

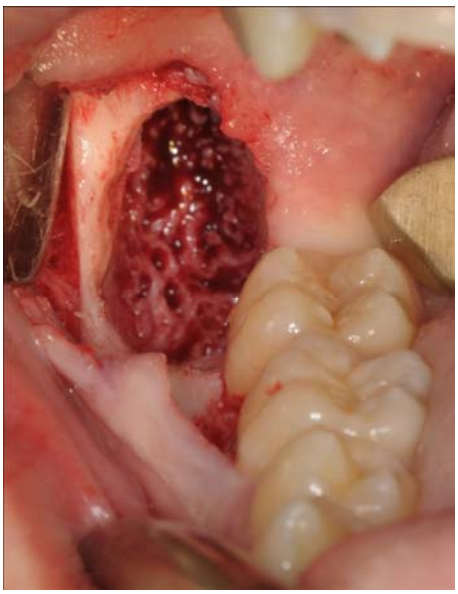


Fig. 3 − Clinical view showing the rough surface of active bone deposition favored by decompression.

All cystic lesions showed a complete osseous regeneration after enucleation, without complications. The mean duration of follow-up was 3.41 ± 1.9 years, and no recurrences were noted.

Discussion

Childhood is characterized by more frequency of developmental odontogenic cysts [1], what is in contrast with the adult period [2, 9], dominated by inflammatory cysts. This could be explained by the fact that in pediatric age the maxillamandibular complex is characterized by active growth as well as development of primary and permanent dentition. All that can be correlated with the appearance of developmental cysts [1, 2]. Therefore, cysts are fast growing, symptomless and usually diagnosed when they reach a considerable size, which is in agreement with our findings.

When it comes to the pediatric age the literature does not present any consensus on a uniform treatment plan for the jaw cystic lesions, especially the large ones. The primary goal of jaw cysts treatment is their complete surgical removal in order to avoid any further lesion growth, large bone defects formation, frequent infections, malignant alteration and recurrences. Therefore, the treatment of choice would generally be enucleation [9, 10]. Sometimes however, this approach can jeopardize secondary objectives, such as preservation of adjacent important anatomical structures, vitality of affected teeth and most notably the teeth germs [10]. Moreover, enucleation of large cystic lesions is inevitably followed by creation of large bone defects impairing complete bone regeneration. All these considerations point to the importance of selecting less aggressive treatment modality for cystic lesions in childhood.

Although cystic lesion larger than 3 cm^2 are usually indicated for decompression [11] this study encompassed three lesions smaller than 3 cm^2. Nevertheless, all treated lesions were more than 2 cm in diameter, which is considered to be large cystic lesion in children [12], especially concerning small dimensions of alveolar ridges. Removal of these lesions in a single-stage procedure would compromise important goals of treatment (preservation of adjacent important anatomical structures and vitality of the affected teeth), making decompression as more convenient treatment modality.

In 92% of dentigerous cysts shrinkage completely occurred, so decompression became the only and final treatment. Additionally, 85.7% of impacted teeth were spontaneously arranged in physiologically determined position within dental arches. These results indicate a two-fold effect of decompression. First of all, there is a great capacity of bone regeneration in pediatric age thus simultaneously providing spontaneous eruption of affected permanent teeth. At the same time, decompression guided tooth eruption encouraged successful bone regeneration, avoiding second stage surgery and orthodontic treatment (Figure 2). Complete disappearance of lesion was probably possible due to creeping substitution by normal mucosa from the edges of the lesion growing in and replacing the cystic epithelium [13]. In that way, decompression spontaneously turns into marsupialization, making this a definitive therapy. Our finding is consistent with the report of Anavi et al. [8], showing that decompression may serve as the primary treatment of non-aggressive cystic lesions. Previous reports also emphasized that marsupialization as conservative treatment modality would be a favorable treatment of choice for extensive dentigerous cysts in children [14, 15].

Our study showed only one case where, following decompression, enucleation of dentigerous cyst was necessary. A potential reason for this could be a complex cyst shape not allowing appropriate irrigation of its entire lumen. Besides, position and complete root formation of impacted tooth might interfere with shrinkage, as well. On the other hand, the literature provides information that dentigerous cysts were most frequently treated with enucleation and extraction of the impacted teeth, while efforts were made to preserve usually the canines [9, 10]. Moreover, if enucleation of extensive dentigerous cyst is performed in the first place it would lead to the loss of several teeth with potential functional, cosmetic and psychological consequences [10].

Our results highlight the fact that decompression is effective in promoting very high percentage of teeth preservation (92.8%), where in 85.7% of cases the teeth erupted spontaneously, while orthodontic traction had to be used only in one (7.1%) patient. A probable reason for this high success rate might be the consequence of unfinished root formation, which gives the teeth strong eruptive power. A similar observation was made by Qian et al. [16], who found that age, gender, tooth angulation and cusp depth were not significant risk factors for the eruption of the impacted teeth, but rather the level of root formation which might play an important role. On the other hand, Yahara et al. [17] found that patient's age may affect successful eruption of premolars in dentigerous cyst. The main age with successful eruption was in agreement with the age in our survey, while the main age of patient of the non-erupted group was significantly older. Two patients in our survey, the one with orthodontic traction and the only one in whom tooth was extracted, were elder

and with completely formed roots, so spontaneous eruption could not have been accomplished. Moreover, it was premolar that was extracted, due to dental crowding and in agreement with orthodontist.

Unlike dentigerous cysts, all KCOTs were treated with two-stage surgical approach − decompression followed by enucleation in the second act. This is due to KCOT's characteristic aggressive behavior and a remarkably high recurrent rate, up to 56% [18]. Even though successful marsupialization of KCOT was reported in the literature [6, 13], it is considered that for aggressive lesions secondary definitive surgery should be performed [8, 11, 19]. Since our results show that decompression time for reducing the half size of KCOT lasted from 6 to 16 months (Table 1), it would take much longer time for lesions to completely disappear, especially because the increase in bone density together with the decrease in cyst volume is reported to be the highest in the first 3 months [20]. This is why we reduced a long decompression/marsupialisation time, considering it to be the main weakness of the therapy [6, 21] due to the possible malignant alteration of the lesion left in-site [22].

The first strength of new bone apposition favored by decompression (Figure 3) is that the lesion volume was reduced providing conditions for its safer and easier removal. Therefore, none of postoperative complications were recorded in our study. Moreover, smaller bone defect after decompression contributed to complete bone regeneration, observed on all control radiograms one year after the final surgery (Figure 1). Besides, the decompression resulted in preservation of the most of the affected teeth, except for impacted ones, predominately the third molars. Only three teeth the roots of which protruded in the lesion lumen at the time of enucleation had to be extracted. Additionally, because of both bone apposition and eruptive power, the impacted tooth was moved to more suitable position, so extraction was easier to perform with lower surgical trauma and risk of injuring adjacent anatomical structures (Figure 1b).

When it comes to the recurrent rates, we did not find recurrences at all, which is far better than reported in the literature and could be explained by several things. Firstly, this is due to surgical approach combining decompression followed by enucleation with additional use of Carnoy solution. It is well-known that after decompression the keratocystic wall becomes thickened and more cohesive, which makes it easier for complete removal [21]. Besides, previous studies have found that decompression favors epithelial transformation into its less aggressive form (orthokeratinized epithelium) or into epithelium of normal oral mucosa [6, 13, 21, 23]. Moreover, every histopathologically proven KCOT was additionally treated with Carnoy solution after excision of the lesion. The use of this was proven to successfully reduce the recurrence rate [24, 25] by destroying epithelial residues of the cyst wall that may have been left behind in the bone defects after the enucleation alone [26]. In addition, all impacted and teeth which roots protruded in the lesion lumen at the time of enucleation were extracted thus minimizing the chance for recurrence due to the possible presence of daughter cysts in the periodontal ligament and attached gingiva of affected teeth [27]. On the other hand, our mean follow-up period was relatively short [3.88 ± 2.75 (from 2 to 8) years]. Forssell at al. [28] found only 3% of KCOT's recurrences in the first year after the surgery, while within three years postoperatively, the recurrence rate rose up to 37%. According to the literature, recurrences usually appear within the first 5 years [25, 27, 29]; so it seems a that longer follow-up (5 years) would give more realistic results.

Regarding decompression itself, the mean decompression time in our survey was 8.2 months regardless the type of cystic lesion. These findings are in line with the results of Anavi et al. [8] who pointed out that intensive skeletal growth during children's age was probably responsible for a shorter decompression time and a higher reduction rate when the results were compared to adults. In addition, our results show a significantly shorter decompression time for dentigerous cyst than for KCOT. On the contrary, Kubota et al. [30] found that there was no difference between the relative speed of shrinkage of dentigerous cysts and KOCT. In their study the average age of the patients was 45 years and it is quite clear that their impacted teeth had definitely lost the growth potential, what is totally different from the high grow potential in childhood. Our findings also show no significant difference between the mean decompression time for maxillary and mandibular lesions, probably due to the excellent blood supply and the ongoing growth process of pediatric patients.

Conclusion

Within the limits of this study, we conclude that large dentigerous cysts can be successfully treated with decompression as the definite therapy method. This simple and minimally invasive approach, highly appropriate in children, provides complete bone regeneration and spontaneous eruption of the affected permanent teeth; KCOTs in children require the two-staged surgical approach − decompression followed by enucleation in the second act. Decompression of large KCOTs in children provides lower surgical trauma, easier access for extraction of impacted teeth, reduced recurrent rate and good conditions for complete bone regeneration. At the same time, general anesthesia is avoided; regenerative capacity of both jaws are similar in pediatric age.

Aknowledgement

This study was supported by the Serbian Ministry of Education, Science and Technological Development, Grant N°, 175021.

REFERENCES

1. *Bodner L.* Cystic lesions of the jaws in children. Int J Pediatr Otorhinolaryngol 2002; 62(1): 25−9.
2. *Manor E, Kachko L, Puterman MB, Szabo G, Bodner L.* Cystic lesions of the jaws - a clinicopathological study of 322 cases and review of the literature. Int J Med Sci 2012; 9(1): 20−6.
3. *Sun KT, Chen MY, Chiang HH, Tsai HH.* Treatment of large jaw bone cysts in children. J Dent Child (Chic) 2009; 76(3): 217−22.
4. *Hyun H, Hong S, Kim J.* Recurrent keratocystic odontogenic tumor in the mandible: a case report and literature review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108(2): 7−10.
5. *Takeda Y.* Hyaline bodies and secondary dental cuticle in dentigerous cyst. J Oral Pathol 1985; 14(3): 268−9.
6. *Nakamura N, Mitsuyasu T, Mitsuyasu Y, Taketomi T, Higuchi Y, Ohishi M.* Marsupialization for odontogenic keratocysts: long-term follow-up analysis of the effects and changes in growth characteristics. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 94(5): 543−53.
7. *August M, Faquin WC, Troulis MJ, Kaban LB.* Dedifferentiation of odontogenic keratocyst epithelium after cyst decompression. J Oral Maxillofac Surg 2003; 61(6): 678−83.
8. *Anavi Y, Gal G, Miron H, Calderon S, Allon DM.* Decompression of odontogenic cystic lesions: clinical long-term study of 73 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112(2): 164−9.
9. *Iatrou I, Theologie-Lygidakis N, Leventis M.* Intraosseous cystic lesions of the jaws in children: a retrospective analysis of 47 consecutive cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 107(4): 485−92.
10. *Motamedi MH, Talesh KT.* Management of extensive dentigerous cysts. Br Dent J 2005; 198(4): 203−6.
11. *Gao L, Wang X, Li S, Liu C, Chen C, Li J, Zhi K.* Decompression as a treatment for odontogenic cystic lesions of the jaw. J Oral Maxillofac Surg 2014; 72(2): 327−33.
12. *Bodner L, Woldenberg Y, Bar-Ziv J.* Radiographic features of large cystic lesions of the jaws in children. Pediatr Radiol 2003; 33(1): 3−6.
13. *Pogrel AM, Jordan RC.* Marsupialization as a definitive treatment for the odontogenic keratocyst. J Oral Maxillofac Surg 2004; 62(6): 651−5.
14. *Hu Y, Chang Y, Tsai A.* Conservative treatment of dentigerous cyst associated with primary teeth. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011; 112(6): 5−7.
15. *Koca H, Esin A, Aycan K.* Outcome of dentigerous cysts treated with marsupialization. J Clin Pediatr Dent 2009; 34(2): 165−8.
16. *Qian W, Ma Z, Xie Q, Cai X, Zhang Y, Yang C.* Marsupialization facilitates eruption of dentigerous cyst-associated mandibular premolars in preadolescent patients. J Oral Maxillofac Surg 2013; 71(11): 1825−32.
17. *Yahara Y, Kubota Y, Yamashiro T, Shirasuna K.* Eruption prediction of mandibular premolars associated with dentigerous cysts. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 108(1): 28−31.
18. *Blanas N, Freund B, Schwartz M, Furst IM.* Systematic review of the treatment and prognosis of the odontogenic keratocyst. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90(5): 553−8.
19. *Kaczmarzyk T, Mojsa I, Stypulkowska J.* A systematic review of the recurrence rate for keratocystic odontogenic tumour in relation to treatment modalities. Int J Oral Maxillofac Surg 2012; 41(6): 756−67.
20. *Zhao Y, Liu B, Han Q, Wang S, Wang Y.* Changes in Bone Density and Cyst Volume After Marsupialization of Mandibular Odontogenic Keratocysts (Keratocystic Odontogenic Tumors). J Oral Maxillofacial Surg 2011; 69(5): 1361−6.
21. *Marker P, Brøndum N, Clausen PP, Bastian* HL. Treatment of large odontogenic keratocysts by decompression and later cystectomy: a long-term follow-up and a histologic study of 23 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1996; 82(2): 122−31.
22. *Falaki F, Delavarian Z, Salehinejad J, Saghafi S.* Squamous cell carcinoma arising from an odontogenic keratocyst: a case report. Med Oral Patol Oral Cir Bucal 2009; 14(4): 171−4.
23. *Pogrel AM.* Treatment of keratocysts: the case for decompression and marsupialization. J Oral Maxillofac Surg 2005; 63(11): 1667−73.
24. *Gosau M, Draenert FG, Müller S, Frerich B, Bürgers R, Reichert TE*, et al. Two modifications in the treatment of keratocystic odontogenic tumors (KCOT) and the use of Carnoy's solution (CS)—a retrospective study lasting between 2 and 10 years. Clin Oral Invest 2009; 14(1): 27−34.
25. *Stoelinga PJ.* The treatment of odontogenic keratocysts by excision of the overlying, attached mucosa, enucleation, and treatment of the bony defect with carnoy solution. J Oral Maxillofac Surg 2005; 63(11): 1662−6.
26. *Stoelinga PJ.* Long-term follow-up on keratocysts treated according to a defined protocol. Int J Oral Maxillofac Surg 2001; 30(1): 14−25.
27. *Tolstunov L, Treasure T.* Surgical treatment algorithm for odontogenic keratocyst: combined treatment of odontogenic keratocyst and mandibular defect with marsupialization, enucleation, iliac crest bone graft, and dental implants. J Oral Maxillofac Surg 2008; 66(5): 1025−36.
28. *Forssell K, Forssell H, Kahnberg KE.* Recurrence of keratocysts. A long-term follow-up study. Int J Oral Maxillofac Surg 1988; 17(1): 25−8.
29. *Pitak-Arnnop P, Chaine A, Oprean N, Dhanuthai K, Bertrand J, Bertolus C.* Management of odontogenic keratocysts of the jaws: A ten-year experience with 120 consecutive lesions. J Craniomaxillofac Surg 2010; 38(5): 358−64.
30. *Kubota Y, Imajo I, Itonaga R, Takenoshita Y.* Effects of the patient's age and the size of the primary lesion on the speed of shrinkage after marsupialisation of keratocystic odontogenic tumours, dentigerous cysts, and radicular cysts. Br J Oral Maxillofac Surg 2013; 51(4): 358−62.

Received on August 28, 2014.
Revised on November 19, 2014.
Accepted on December 8, 2014.
Online First December, 2015.

ORIGINAL ARTICLE

UDC: 615.851
DOI: 10.2298/VSP140911141H

Therapist's interpersonal style and therapy benefit as the determinants of personality self-reports in clients

Način ophođenja terapeuta prema klijentima i korist od psihoterapije kao odrednice samoprocene ličnosti klijenata

Nina Hadžiahmetović, Sabina Alispahić, Đenita Tuce, Enedina Hasanbegović-Anić

Department of Psychology, Faculty of Philosophy, University of Sarajevo, Sarajevo, Bosnia and Herzegovina

Abstract

Background/Aim. In (counter)transference relationship therapist's interpersonal style, implying the perceived relation of therapist to a client (patient) in terms of control, autonomy, care and positive feedback, has been shown to be important. The aim of our study was to assess the relationship between therapist's interpersonal style and clients' personality self-reports. Within therapist's interpersonal style, preliminary validation of the Therapist's Interpersonal Style Scale has been conducted, which included double translation method, exploratory factor analysis, confirmatory factor analysis, as well as the reliability tests of the derived components. **Methods.** This research was conducted on a group of 206 clients, attending one of the four psychotherapy modalities: psychoanalysis, gestalt therapy, cognitive-behavioral and systemic family therapy. Beside Therapist's Interpersonal Style Scale, Big Five Questionnaire and Therapy Benefit Scale were administered, showing good internal consistency. **Results.** Principal component analysis of therapist's interpersonal style singled out two components Supportive Autonomy and Ignoring Control, explaining 42% of variance. Two-factor model of the therapist's styles was better fitted in confirmatory factor analysis than the original 4-factor model. Structural model showing indirect and direct effects of therapist's interpersonal styles on self-reports in clients indicates good fitness ($\chi^2_{(12)} = 8.932$, $p = 0.709$; goodness-of-fit index = 0.989), with Ignoring Control having direct effect on Stability, Supportive Autonomy on Therapy Benefit, and Therapy Benefit on Plasticity. **Conclusion.** The results of this study indicate the importance of further research on therapist's interpersonal style, as well as further validation of the instrument that measures this construct. Besides, a client's perception that the therapy is being helpful could instigate more explorative and approach-oriented behavior, what indirectly might contribute to a client's stability.

Key words:
psychotherapy; physician-patient relations; personality; personality assessment; questionnaires.

Apstrakt

Uvod/Cilj. U (kontra)transfernom odnosu značajan je stil terapeuta, koji podrazumeva poimanje relacije klijenta sa terapeutom u smislu kontrole, autonomije, brige i pozitivne povratne informacije. Cilj našeg istraživanja bio je ispitivanje povezanosti interpersonalnog stila terapeuta i samoprocene ličnosti klijenta. U okviru načina ophođenja terapeuta prema klijentima sprovedeno je i prethodno vrednovanje skale načina ophođenja terapeuta prema klijentima koje je obuhvatilo metod dvostrukog prevoda, eksploratornu faktorsku analizu, konfirmatornu faktorsku analizu i ispitivanje pouzdanosti izdvojenih faktora. **Metode.** Istraživanje je rađeno na grupi od 206 klijenata, koji su bili na psihoterapiji primenom jednog od četiri psihoterapijska modaliteta: psihoanalize, geštalt terapije, kognitivno-bihverioralne i porodične sistemske terapije. Pored skale interpersonalnog stila terapeuta, primenjene su i skala procene za Velikih pet i skala percepcije koristi psihoterapije, sa zadovoljavajućom unutrašnjom stabilnošću. **Rezultati.** Analizom glavnih komponenti načina ophođenja terapeuta izdvojena su dva faktora, suportivna autonomija i ignorišuća kontrola, koji objašnjavaju 42% varijanse. Model načina ophođenja terapeuta sa dva faktora pokazao je bolje uklapanje u konfirmatornu faktorsku analizu od originalnog modela četiri faktora. Strukturalni model, koji prikazuje direktne i indirektne efekte načina ophođenja terapeuta na samoprocenu ličnosti klijenta pokazuje dobru podešenost ($\chi^2_{(12)} = 8{,}932$, $p = 0{,}709$; *goodness-of-fit index* = 0,989), pri čemu ignorišuća kontrola direktno doprinosi stabilnosti, suportivna autonomija percepciji koristi terapije, a percepcija koristi terapije plastičnosti. **Zaključak.** Rezultati ove studije upućuju na značaj daljeg istraživanja načina ophođenja terapeuta prema klijentima i vrednovanja instrumenta kojim se meri ovaj odnos. Pored toga, osećaj klijenta da je terapija korisna mogao bi potaknuti više istraživačkog ponašanja i ponašanja orijentisanog na cilj, što bi indirektno moglo doprineti stabilnosti klijenta.

Ključne reči:
psihoterapija; lekar-bolesnik odnosi; ličnost; ličnost, procena; upitnici.

Correspondence to: Nina Hadžiahmetović, Faculty of Philosophy, University of Sarajevo, Franje Račkog 1, Sarajevo, Bosnia and Herzegovina. E-mail: n.hadziahmetovic@gmail.com

Introduction

Self-determination theory (SDT) [1], the theory of basic psychological needs promotes autonomy as a sense of volition and psychological freedom [2], what is of quintessential significance for client satisfaction in the process of psychotherapy. Clients have differential motivations for therapy that is susceptible to change depending on external factors such as therapist's interpersonal style (TIS) [3]. Therapist's style can be self-determination oriented when promoting support, involvement and information, or controlling, when manifested as the opposite. Recent studies have come to interesting discovery that it is not the visible and superficial trademarks of a therapist, such as sociodemographic variables, professional experience or sex, the level of training and type of orientation that contribute to therapeutic outcome [4, 5] as expected, what directs researchers to pay more attention to therapist-client matching [4]. The therapist-client communication varies from autonomy to paternalism [6]. The more perceived coercion increases, the more positive evaluation of therapeutic relationship decreases [7]. Clients reported being more intrinsically motivated when therapist provided the opportunity for them to make a decision, expressed sincere care, provided constructive feedback or did not exert pressure for specific activities, and more amotivation was evident, when therapists were controlling [3]. In a meta-analysis of the pooled data on interaction styles, including control and negotiation as option, caring interaction style (e.g. sensitive, friendly, relaxed and open) had a moderate and positive correlation with satisfaction with consultation [6]. Personal therapeutic attributes that turned out to positively impact therapeutic alliance include an array of characteristics such as conveying a sense of being trustworthy, affirming, interested, alerted, affiliative type behavior as helping and protecting, coherent communication style, and attunement to patient [8]. As addition, active, engaging and extraverted therapists produced faster symptom reduction in short-term therapy, but also non-intrusive therapists generated better outcome in long-term therapy within the range of 3 years of follow-up [9]. In a research of the client-oriented existential therapy failure, the main factors of negative outcome were the lack of therapeutic attunement and inflexibility [10]. In another research in the domain of different psychoanalytic orientation, technical adherence and directivity was shown in the therapists with hostile and controlling introjects. These kinds of therapists were most likely to monitor their own behavior as control for potential external disapproval of their skills [11]. But not all researches argue against control in therapy. Taking into account cultural framework, Chinese clients perceive directive therapist's style to be the most effective, finding concrete homework to be more useful than only talking to therapist. Leading conversation guided by the therapist was also considered to be appropriate and the rest focused on therapist-client match, where therapists were regarded as someone who needed to know how to click with others [12]. The question whether clients benefit from directive counseling is yet to be addressed, since controlling does not necessarily subsume coercion. It most often relates to a structure, especially if promoted in a rather autonomy-supportive manner [2]. When expressed as a support, recommendation is likely to be experienced as informational, leaving the client to make a decision for him/herself [13]. Whether it is autonomy or control in therapy that matters, therapist-client matching has been stressed out on numerous occasions. A study shows that clients who were matched with their preferred treatments had a 58% chance of outcome improvement, so it is recommendable to include client preferences into treatment [14].

Even though every therapy has its own effectiveness criteria, the measure of client satisfaction was introduced as the part of the broader scope approach to assess the quality of service and some of previous client satisfaction measures encompassed subscale ranging from relevance (fitting the service with the problem), impact (effect of services on the problem) and gratification (effect of service on client's self-efficacy) [15].

Aside from attending therapy and therapist's characteristics, the great deal of research indicates personality dispositions to best predict personal wellbeing. The findings of positive contribution of personality are very consistent [16, 17]. As stated by Steel et al. [18], personality and wellbeing have much stronger correlation than previously recognized. Studies consistently show extraversion to have a positive and neuroticism to have a negative influence on wellbeing with spillover effect on the overall wellbeing. Findings reveal genetic dispositions in personality and long-lasting influence of personality has been shown in longitudinal studies [19–22]. Basic personality traits are described through the Big Five dimensions, replicable independently of culture: neuroticism, extraversion, openness to experience, agreeableness and conscientiousness. Albeit there is a certain amount of published papers contending these traits are structurally organized into two higher-order factors, usually labeled stability (neuroticism, that is emotional stability, agreeableness and conscientiousness) and plasticity (extraversion and openness to experience) [23] with underlying biological substrates, Ashton et al. [24] argue this could just be a methodological artifact, representing two or more blends of the Big Five factors.

Scholarly attention has been paid so far to the therapeutic matching and alliance itself, but it appears that the quality of the alliance is more the result of therapist's actions or characteristics playing the most important role in achieving beneficial outcome, since only the variability within therapist and not the client was significantly predictive of outcome, as the recent study shows [25]. Furthermore, it is also still unknown what clients think is important for psychotherapy [12]. In view of previous finding advising not to interpret heritability of personality as the impossibility to change [19], this research included personality as the outcome variable; especially since the traits organized as higher-order factors named stability and plasticity may represent socially desirable self-presentation behavior [24]. Since the personality as described could be the protective or impairing factor to the wellbeing by itself, it was interesting to examine whether these features are at least to a certain degree susceptible to be determined by the exerted therapist's style.

Therapist's style has previously been operationalized as the four-scale construct [3] (including support of autonomy, control, care, and support of competence) pertaining to interpersonal behaviors with the function of motivational antecedents. This scale was originally adapted from Pelletier et al. [26] where it was first administered to capture motivational antecedents in sports. It was shown that autonomy supportive behaviors providing opportunities for choice foster intrinsic motivation, while the coach's behaviors manifesting the lack of care for the athlete undermine self-determined motivation [26]. Applied to the psychotherapy context, the same pattern of results occurred showing the perception of the therapists as providing opportunity to make decisions, carrying for clients, giving constructive feedback, or not putting pressure on clients to be related to self-determined motivation toward therapy [3]. No previous studies to our knowledge considered contribution of TIS to personality self-reports. Therefore, the general aim of this study was to assess the contribution of therapist-relevant variables, TIS specifically, to the Big Five personality self-report in clients. In light of what is known of interpersonal style in general, we were interested to examine the direct and indirect effect of extracted therapist's styles on personality self-report. Indirect effect was assessed through therapy benefit. The latter was invoked as the mediator into the model, as the measure of satisfaction with therapy, since interpersonal style is usually regarded as motivational antecedent and therapy satisfaction as motivational consequence [3].

However, since TIS was previously measured by *ad hoc* constructed scale (TIS) for the purpose of motivation for therapy scale validation, without previous history of a thorough psychometric validation *per se*, the first and foremost purpose of this study was to preliminary validate this scale into Bosnia and Herzegovina (BH) languages. We were first interested in the translation of scale from the original English into BH languages, then to conduct the double translation procedure, which was followed by exploratory and confirmatory analyses of TIS structure, as well as the reliability testing of the extracted factors.

In previous research [26], it was recommended that the degree to which parents and coaches adhere to supporting the children and spending time with them should facilitate self-determination in children. We are not certain whether the patterns of behaviors pertaining to coach or parents (specifically the ones including spending time or providing permanent feedback) are totally applicable to the patterns of the therapist's behavior, especially since many therapy schools have different rationale as to how to approach a client. For this reason, and being aware that the 4-factor structure has not been confirmed yet, we did not make any definite assumptions regarding the preset number of factors to be extracted. But we did expect that if autonomy/support prone styles were extracted, these should have positive, and controlling prone styles, also in case of exploratory extraction, should have negative contribution to therapy benefit and personality self-report, the latter defined by two presumably [24] secondary factor loaded personality variables Stability and Plasticity.

Methods

The study sample included 206 clients (154 females, 47 males, 3 participants did not specify their gender; mean age 33.99 ± 10.17), attending 4 psychotherapy schools: psychoanalysis (n = 28), gestalt therapy (n = 76), cognitive-behavioral therapy (n = 75) and systemic family therapy (n = 27). Most of clients had university degree (n = 109), following high school diploma (n = 84), year degree (n = 12) and only one participant completed primary school. As for employment status, 82 clients had full-time job, 57 were unemployed, 23 had part-time employment, 21 fixed term employment, 7 were retired and 16 did not provide information about their employment. With respect to marital status, 89 clients lived in formal marriage or with a partner, 84 were never married, 25 were divorced and 1 widowed. The rest 7 did not provide information in reference to their marital status. Beside therapy in which 140 clients were enrolled without taking medication, 65 clients also had joint medication treatment and one left out the information about medication intake.

In order to assess TIS and personality traits, two self-report measures were administered: The Adapted Therapist's Interpersonal Style Scale and the Big Five Questionnaire [27].

The first instrument was adapted for the purpose of this research. The fundament upon which the adaptation was made was the original TIS scale [3]. The scale was originally constructed in English and is made of 4 subscales, consisting of 3 items each, aimed to assess 4 different types of interpersonal styles. The 12 items forming 4 subscales, originally adapted from Pelletier et al. [26], include Support of Autonomy (e.g. "My therapist provides me with opportunity to take personal decisions"), Control (e.g. "My therapist pressures me to do what he/she wants."), Care and Support of Competence. The answers are given on a Likert type scale ranging from 1 to 7. TIS Scale was originally adapted, although not psychometrically validated, from the similar scale administered in sports domain, the Coach's Interpersonal Style (CIS) [26]. The latter consisted also of four scales: Autonomy Supportive Climate (e.g. "My coach accepts that mistakes I make are part of a learning process."), Caring (e.g. My coach cares about me."), Providing Structure (e.g. When my coach asks me to do something, he or she gives me a rationale for doing it."), and Competence Feedback (e.g. "The feedback I receive from my coach is constructive in helping me make improvements."). Sample items in both scales are very similar, except that the "coach" was switched by the "therapist" to accommodate more to the therapist's style.

The adapted TIS scale was first translated from English into BH languages by two psychologists in clinical domain. Then back translation process was conducted in which bilingual English/BH language(s) speaking psychologist independently translated the BH version of the scale back to English. This translation was again thoroughly checked by English professor. The back translation process was followed by double translation procedure. It included sending the back translation version of the instrument to the author of the original scale [3] for further confirmation about the equivalence of the original and the translated items. After the check of double translation it turns out that 7 items were identical in meaning as in the original TIS, while

the rest 5 items were slightly or considerably changed, and different in meaning comparing to the original TIS. Analyzing the rest 5 items by its content, it was noticeable that changes were made in a more control and problem-solving direction, since our study was more focused on autonomy or control manifesting therapist's behavior in general. So we did not further consider our instrument we called the Adapted TIS Style Scale to be the equivalent to the original TIS, but we conducted a psychometric validation on our version of the instrument to establish whether it is in its own right applicable in other analyses. This process we discuss more in the Discussion section and the adapted version of the instrument is given in the Appendix. In this research, two factors pertaining to therapist's styles into which all items were aggregated, were singled out, what is explained in more detail in the Results section. The factors showed, though not perfect, acceptable reliability of the subscales: Control (Cronbach's alpha 0.63) and Autonomy (Cronbach's alpha 0.78).

The Big Five Questionnaire consists of 50 adjective items on a 5-point Likert scale forming 5 subscales intended to capture the Big Five personality traits: emotional stability (inverted Neuroticism), extraversion, intellect (openness to experience), pleasantness (agreeableness) and conscientiousness. The subscale reliabilities in this research were very good ranging from 0.82 to 0.87.

Therapy Benefit Scale consists of 3 very simple questions measuring the satisfaction with therapy: "To what extent is the therapeutic treatment you are currently involved in important for you?", "How much do you consider therapy helps you?", and "To what extent are you satisfied with the therapy you are involved in?" All questions were responded on a 4-point Likert scale adding up to a total score of therapy benefit. Principal component analysis revealed all items to load on one factor, enabling to add individual responses to a common score. Besides, reliability of this measure, in spite of containing only 3 items, was very good (Cronbach's alpha 0.79).

All data were collected on a voluntary basis, respecting the anonymity of clients. Clients gave consent to participate in the study and could withdraw from research at any point. They were already enrolled to therapy for a substantial time to be able to evaluate their perception of the relationship with the therapist. Prior to the questionnaire distribution, the therapists of the four above mentioned therapy schools were contacted to recruit interested clients into the research. The questionnaires sealed in envelopes were mailed to different locations in BH. Clients were given the questionnaires they opened on the site, filled it in the waiting-room with no suggestions from the therapist, sealed the filled forms again, and handed it to the therapist whereupon they were returned to the researcher.

Data were analyzed using Principal Component Analysis (PCA) to derive components of the therapist's interpersonal style. The components derived in exploratory factor analysis (EFA) were also fitted for confirmation in subsequent confirmatory factor analysis (CFA). Correlational analysis was engaged to examine whether therapist's styles have any relations to personality self-report measures. Finally, structural equation modeling was conducted to shed further light on the direct and indirect effects of therapist's styles on personality self-report as mediated through therapy benefit, as well as to examine secondary factor loadings personality model. Descriptive analyses are also provided in the following section. To obtain the analyses, two statistical software packages were used: IBM SPSS Statistics for Windows, Version 19.00 and IBM SPSS Amos, Version 19.00.

Results

To assess how many components would be suitable to be extracted in order to best explain the variance of the interpersonal therapist's styles, PCA was conducted (Table 1).

Table 1

Principal Component Analysis (PCA) Pattern Matrix of the items

Items	Supportive Autonomy	Ignoring Control	h^2
3. My therapist gives me the feedback about the way I make a progress.*	**0.87**	0.18	0.69
1. The feedback I receive from my therapist is constructive in helping me make improvements.	**0.86**		0.72
8. My therapist consults me before (s)he decides how to address my problems.*	**0.80**	0.10	0.59
11. My therapist provides me with lots of opportunities to take personal decisions in what I do.	**0.55**	-0.12	0.37
2. When I ask my therapist to help me solve a problem, he or she asks me what I think before giving me his or her opinion.	**0.48**	-0.19	0.33
10. I feel that my therapist doesn't care how much I improve through therapy.	**-0.20**		0.05
7. My therapist pressures me to do what he or she wants.	0.11	**0.76**	0.53
4. My therapist is trying to impose her/his ideas on me.*		**0.66**	0.44
6. I feel that my therapist is indifferent towards me.	-0.12	**0.61**	0.44
12. My therapist leaves me with little choice about the ways in which my problems could be resolved.*	-0.10	**0.55**	0.35
5. The feedback I get from my therapist is basically useless criticism.	-0.31	**0.44**	0.38
9. **My therapist is being harsh to me.***		**0.44**	0.18

Note: The items written in boldface are changed and adapted in translation from English to BH languages, so due to a change, they are left in the translated form. Other items are written as in the original Therapist's Interpersonal Style.

Firstly, we tried to conduct PCA with 4 preset factors, as in the original TIS Scale to examine whether 4 subscales can be singled out. By applying first the default orthogonal rotation, since there were no similar previously published results upon which to build our analysis, it was evident from the values in component transformation matrix that in fact 4 factors were intercorrelated. The 4-factor solution is by the predetermined extraction rationale the solution that best corresponds to the Kaiser-Guttmann's criterion [28] for extraction. However, this solution was not substantiated in structure matrix where only two items saturated component in some cases. The same pattern of results occurred independently from the type of rotation. But, the general criteria in analysis for extraction were fulfilled [28] including Kaiser-Meyer-Olkin (KMO) = 0.731 and Bartlett's Test of Sphericity $\chi^2_{(66)} = 570.75$, $p < 0.001$, indicating that manifest items are correlated to a certain degree to be able to capture a latent component, but not as singular as not to be discernable as distinct entities.

Another criterion for extraction is shown in Figure 1. Cattell's Scree test shows that 2 distinct components probably best explain the latent structure of the therapist's interpersonal style with eigenvalue far exceeding 1. Even though on the basis of visual inspection it is visible that 4 components could be extracted, what corresponds to the Kaiser-Guttmann's extraction criterion as well, it is also visible that the last 2 factors exceeding 1 on the ordinate explain the variance far less than the first 2. Besides, the point of infexion happens on the third component, what makes this component a surplus [28], which is usually not retained in analysis. This is the reason why we actually chose to explore the latent structure of the therapist's style if we suppose 2 intercorrelated components for extraction applying direct oblimin rotation on the components.

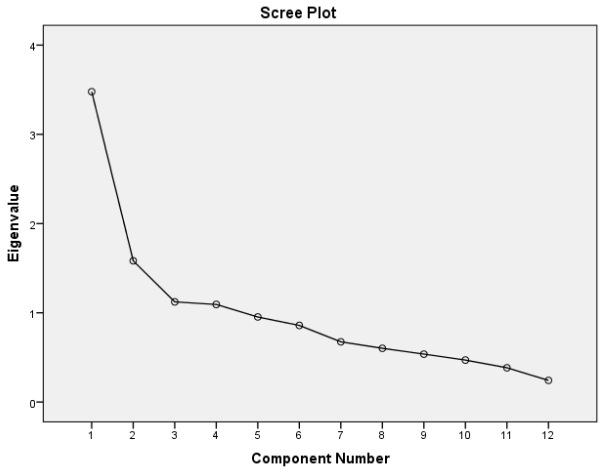


Fig. 1 – Cattell's Scree Test for component extraction.

After preliminary check for the appropriate number, 2 components have been extracted, accounting for 42.17% of variance. The first component labeled Supportive Autonomy explained 28.98% and the second labeled Ignoring Control explained 13.19% of variance. Both components had acceptable reliabilities as outlined in instrument description, even though ignoring control had a bit lower Cronbach's alpha reliability value of 0.63. It is stated in the literature that reliability as low as 0.61 could be acceptable when conducting exploratory studies [29], so this component was further retained in the analysis. Reliability analysis was also conducted on the original four subscales, but internal consistency of the 2 derived components outperformed original scales which had the reliabilities ranging from 0.71 for the support of competence to 0.33 for care, which was the least value. Unlike the original TIS having four subscales, in this research all items were aggregated into two plausible components relating to the conglomerate of the four therapist's styles from the original scale. These were labeled Supportive Autonomy, since the items originally belonging to support of competence and support of autonomy all saturated one component. Ignoring Control was labeled upon the conglomeration of the items originally pertaining to the lack of care and control, which in this exploratory analysis all saturated the second extracted component. Supportive autonomy could be described as giving useful feedback, support for independent decision-making, and consultation prior to expressing own opinion. On the contrary, ignoring control is not captured as giving directive instructions and maintaining structure, but more like negative controlling of client and being ignoring and non-empathetic.

To ascertain these results more thoroughly, we decided to further conduct confirmatory factor analysis. This analysis required *post hoc* updates to the original model outlined in Figure 2.

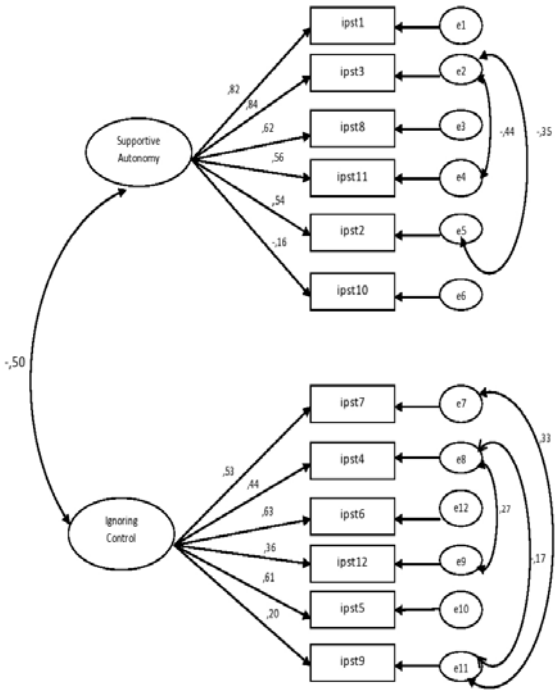


Fig. 2 – Confirmatory model of the Adapted Therapist's Interpersonal Style Scale.

This model was tested for fitting with maximum likelihood method comparing to the 4-factor model as would be in the original scale. The parameters showed, even though the

2-factor model is not fitted on chi-square level $\chi^2_{(48)} = 89.85$, $p < 0.001$ (the significant p means a difference between the observed and default theoretical model) according to which the null hypothesis was rejected, it well outperformed the original model on other parameters. It is also worth noting that χ^2/df was 1.87. The Goodness-of-fit index [30], measuring the fit between the observed and hypothesized covariance matrix (acceptable cut-off over 0.90) for the 2-factor model had a value of 0.935, while other parameters including comparative fit index (CFI), what is recommendable to check [31] and root mean square error of approximation (RMSEA) [32] had the values of 0.923, and 0.065 (PCLOSE = 0.112), respectively, approaching the recommended cut-offs. RMSEA less than 0.05 is usually taken as the indicator of the good model fit [32], which is here almost the case, very closely approaching the desired value. Some of other parameters such as the normed fit index (NFI) [33] = 0.853 argue for the necessary improvement of this model, but considering this analysis to be preliminary and sensitivity of the NFI to the sample size [31], these parameters are not negligible. Unlike the 2-factor model, the 4-factor model showed less favorable parameters as follows: $\chi^2_{(47)} = 121.46$, $p < 0.001$ with much larger χ^2/df ratio 2.58. In the 4-factor model the null hypothesis was also rejected, and the rest of parameters had values farther from acceptable comparing to the posed 2-factor model including: GFI = 0.916, CFI = 0.864, RMSEA = 0.088 the closeness of-fit statistic [(the closeness of-fit statistics PCLOSE) = 0.001), and NFI was even smaller 0.802. What is more interesting, *post hoc* analysis showed that the 4-factor model could be better fitted if we supposed the regression weight on the item 5 ("The feedback I get from my therapist is basically useless criticism".) from Control to be unconstrained, what implies that the item originally belonging to Care has a probable secondary loading on Control, as well. This correlation was pretty high (0.46) for the secondary loading, and was significant ($p < 0.01$). With this modification, the 4-factor model also reached its fitting maximum.

Turning back to the 2-factor model, all estimates were significant, and it also turned out for the extracted components to be inter-correlated ($r = -0.50$, $p < 0.001$), so these components were further retained in other analyses that considered the contribution of interpersonal therapist's styles to personality self-reports.

In the forthcoming analyses, first the correlation of all variables included in structural equation model is shown in Table 2.

In this analysis, the relationship between relevant therapy variables (such as therapist's styles and therapy benefit) and personality self-evaluations was of the greatest interest. As can be observed, the correlations between therapist's styles and therapy benefit are patterned in a predictive way. Supportive autonomy is positively and ignoring control negatively correlated with therapy benefit (Spearman's $rho_{(205)} = 0.50$; Spearman's $rho_{(205)} = -0.39$, respectively, both significant at $p < 0.01$). The correlation between therapist's styles also shows inverse pattern, the more perceived supportive autonomy is expressed, the less perceived ignoring control and *vice versa* (Spearman's $rho_{(206)} = -.44$, $p < 0.01$). Although correlations between personality self-evaluations and therapist's styles are fair, but significant, it is visible that supportive autonomy is almost not at all related to personality, but ignoring control shows fair, and significant correlations to all personality self-reports, Spearman's rho ranging from -0.13 to -0.39. Neuroticism correlates positively with Ignoring Control, Spearman's rho = 0.26 ($p < 0.01$). To obtain the correlations, Spearman's rho as the robust method was engaged for precaution, since few distributions showed a certain asymmetry, especially therapy variables supportive autonomy and therapy benefit being negatively asymmetrical.

To examine the predictability of the relationship between therapist's styles and personality, further regression analyses were conducted, not reported in this research, but they shed further light on potential directionality of the contribution, revealing that it were the therapist's styles, and not the reverse, that contributed to personality self-report. This finding led to setting the structural model to assess the direct and indirect effects of therapist's styles on personality self-report. As outlined earlier, the model was set which included therapist's styles as exogenous variables, therapy benefit as the mediator and personality factors as endogenous variables. The model was also set to be fitted for secondary factor loadings for stability and plasticity, instead of presuming higher-order factor structure. The hypothesized model with the standardized coefficients of the effects is presented in Figure 3.

Table 2

Correlations among the extracted therapist's interpersonal styles, therapy benefit and personality self-evaluations

Scale	1	2	3	4	5	6	7	8
1. Supportive Autonomy	1							
2. Ignoring Control	-0.44**	1						
3. Therapy Benefit	0.50**	-0.39**	1					
4. Neuroticism	-0.10	0.26**	-0.13	1				
5. Extraversion	0.13	-0.14*	0.21**	-0.33**	1			
6. Intellect/Openness to Experience	0.14*	-0.14*	0.17*	-0.35**	0.59**	1	.	
7. Pleasentness/Agreeableness	0.12	-0.13†	0.12	-0.26**	0.19**	0.29**	1	
8. Conscientiousness	0.06	-0.19**	0.10	-0.26**	0.25**	0.39**	0.52**	1
Mean ± standard deviation	29.69 ± 4.77	8.38 ± 3.53	6.87 ± 1.51	32.79 ± 6.94	34.56 ± 8.19	35.46 ± 7.59	42.81 ± 5.56	39.95 ± 6.59

****statistically significant ($p \leq 0.01$); *statistically significant ($p \leq 0.05$); † marginally significant ($p = 0.059$; $p \leq 0.06$).**

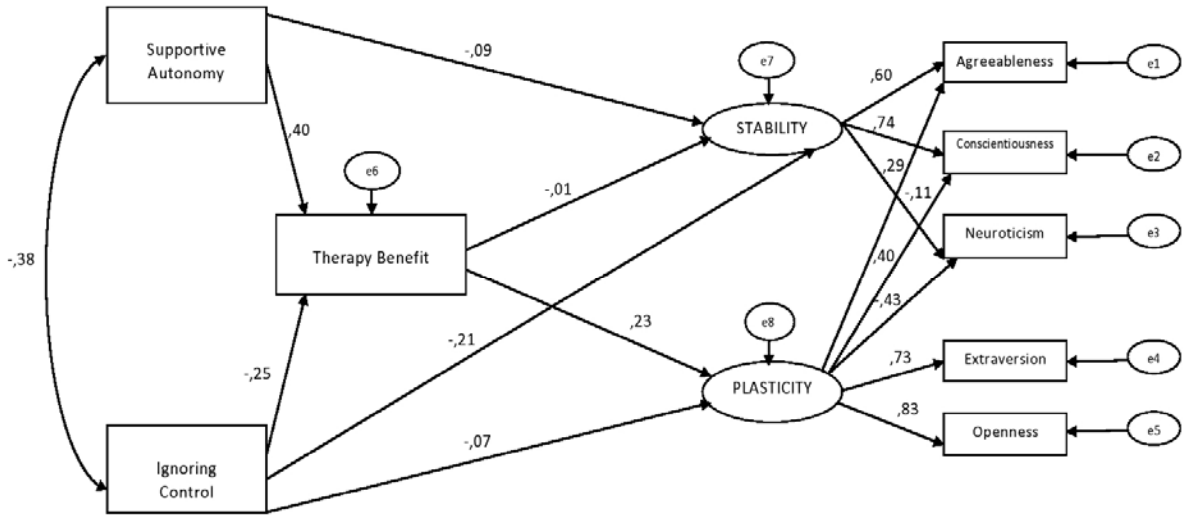


Fig. 3 – The hypothesized structural model of the Therapist's Interpersonal Style effects on personality self-report.

The maximum likelihood model was very well fitted with the following parameters: $\chi^2_{(12)} = 8.93$, $p = 0.709$; GFI = 0.989, RMSEA = 0.00 (the insignificant p here means no difference between the observed and default theoretical model, indicating good fitness of the model). The model provides some direct and indirect effects of TISs to be observed. Supportive autonomy is moderately positively related to therapy benefit ($r = 0.40$, $p < 0.01$) and ignoring control is fairly negatively related to therapy benefit ($r = -0.25$, $p < 0.01$). Supportive autonomy and ignoring control show moderate inverse correlation as two opposite therapist's styles ($r = -0.38$, $p < 0.01$). The interesting finding is that supportive autonomy has no any significant direct effect on either stability (agreeableness, conscientiousness and neuroticism) or plasticity (extraversion and openness), all values approaching 0. On the contrary, ignoring control, though not having direct effect on plasticity, has a direct marginally significant effect on stability ($r = -0.21$, $p = 0.073$). Also indicating of the secondary factor loading in the measurement model is that much of the variance pertaining to stability (agreeableness, conscientiousness and neuroticism) can be explained by Plasticity as the latent variable. As hypothesized, and contrary to the higher-order model, agreeableness and conscientiousness are also explained by plasticity ($r = 0.40$, $r = 0.29$ respectively), independent from being set to regress on stability. And neuroticism is more explained by plasticity ($r = -0.43$), than by stability (-0.11) that was preset in the model. When we analyze standardized indirect effects in more detail, for both supportive autonomy and ignoring control on personality self-reports, mediated by therapy benefit, these effects are very low tending to be zero. Even though therapy benefit has a significant direct contribution to plasticity ($r = 0.23$, $p < 0.01$), the indirect effect both therapist's styles have, mediated by the perception of therapy benefit, is very low. As for the total effects of therapist's styles, the total contribution of ignoring control to plasticity, comparing to supportive autonomy approaching 0, is larger and has the value of -0.13. The total effect of ignoring control on stability (almost completely attributable to direct effect) is also twice as high as for supportive autonomy. Additional important finding, which is in line with the previous assertion that therapist's styles could have contribution to self-presentation in personality, is also a lower total effect on the sole personality traits than on their blends (stability and plasticity), but again, that effect is a bit larger for ignoring control than for supportive autonomy and in range of -0.11, -0.16 and -0.21 for openness, agreeableness and conscientiousness, respectively. All autonomy effects approach 0.

Discussion

Therapeutic relationship and coercion are both important in clinical practice and have driven a lot of attention in the clinical literature [7]. According to some findings [8], alliance is a pan-theoretical construct impacting psychotherapy independently from therapeutic approach. Even though not dealing with alliance by itself, we were interested to examine whether there were some important characteristics of the very therapist that could contribute to the way clients described themselves. Considering the fact that researches such as this are not that common, which is the probable reason for lacking the adequate instruments to measure some concepts, the administration of the Adapted TIS Scale was a pretty challenge. In exploratory factor analysis (PCA) two compo-

nents we called supportive autonomy and ignoring control were derived in which all items, previously belonging to 4 components, were aggregated. Two components extracted in this research had also far better reliabilities than four original subscales, which is also the reason why they were retained in further analysis. Even more so, CFA showed the 2-factor model outperformed the 4-factor model. However, there are a few limitations of the procedure used that should be emphasized. Firstly, some items of the original TIS scale were changed and administered with the changes in translation that were more inclining to what authors considered to be therapeutic language. For instance, item 4 was completely changed corresponding more to the controlling style. Since the original TIS has not to our knowledge been validated before, we are not certain whether 4 factors are confirmed in the first place in other studies, or whether the results would have been different, had we not made changes in the translation process. Furthermore, the fitting indexes for both models show both could be more improved. This is also one of the reasons why the content of the items should be regarded with more care in future studies. We consider our exploratory analysis to be preliminary and certainly, further replications that could contribute to the validation process are advised. Secondly, since there is no explicit theoretical background as for the number of TISs, confirmatory factor analysis is definitely not the method of choice for this confirmation. Being aware of this limitation and considering that our 2-factor model underwent subsequent *post-hoc* modifications introducing covariances between error terms (Figure 2), this confirmatory analysis is in its essence another case of exploration, and not confirmation to be more precise. To be able to fully conduct the process of the validation of TIS scale, some other validity check such as convergent or predictive, which were not specifically considered in this study, should also be introduced in future research. Since interpersonal styles are not only applicable to therapists, as we are aware they were also mentioned in a scale relating to coaches, and probably could be adapted to various domains, more research is needed on how many interpersonal styles are mentioned in other scientific resources and whether all styles could be applied generally, or to only certain domains. Under this rationale the transferability issue of styles studied in coaches to other domains should also be addressed in the future.

Considering the rest of our findings, the unambiguous result was that supportive autonomy had a direct contribution to the perception of therapy benefit, but what happens next and how it affects other outcome variables is yet to be assessed. Contrary to our hypothesis, supportive autonomy did not have any effect on personality variables. Such a result actually coincides with findings that it was the therapist's characteristics that contributed more to the alliance itself than to the outcome, even though some studies report the positive impact of autonomy continued to persist in abstinence behavior long after the treatment was over [13]. We know that behaving in autonomy-supportive manner will not provide any side-effects, but do we know what autonomy essentially is? SDT makes a distinction between autonomy and independence, for autonomy supports a volitional treatment-adherence, while independence implies independent decision-making. In the case of the latter, the opposite would be a total dependence on counselor's direct advices. According to the authors [2], the opposite of autonomy is heteronomy, encompassing free will to adhere whether behavioral changes are induced by internal or external influences. But in this research, we came up with the correlational analysis of -0.38 ($p < 0.01$) between supportive autonomy and ignoring control, implying this is probably not unipolar, but bipolar construct. Other studies also identify autonomy with coercion absence [7]. Since therapeutic alliance is very often given supremacy in the research, the unique contribution of the therapist or the client has often been concealed. But if autonomy as bipolar construct holds truth, then it could be presumed that support is self-understanding in any therapy benefit, but it is probably the active absence of coercion or control what has the impact on the outcome variables. In this research, the reduction of ignoring control, independently from therapy benefit, had positive contribution to stability. We presume that many clients enrolled in therapy with predominant anxiety and depression problems come instable by default, since both of these states are characterized by perceived lack of control to influence external circumstances or one's life. Directive orders without any consultation with a client or treating the client with neglect and lack of care might not be different from the outer therapeutic conditions. Stability also referred to as social propriety or socialization seems to reflect reverted neuroticism, but is also a broader construct for encompassing other two traits [23]. In this model the score of neuroticism, instead of emotional stability, was left purposely, for most of the problems clients come to therapy with, include some sort of neurotic symptoms, so it was important to keep neuroticism as the outcome variable. In other research [34], stability negatively predicted externalizing behavior (correlation -0.71), including aggression, vandalism, drug abuse, opposition and hyperactivity. Considering the results obtained in this research it could be surmised that the lack of control and restraint coming from the therapist could contribute positively to the reduction of behaviors such as aggression and substance abuse, what should be investigated in further research. But one has to keep in mind precaution by not precluding the possibility that it is just the self-presentation restrained ignoring control contributes to and certainly not the change in the trait neuroticism. One has to be aware that this study does not argue for changing the personality as the outcome variable, especially for it being the input variable in many other cases, but rather that might have contribution to the way clients present themselves in the self-report. Other factor that can account for this assumption is the instrument used to assess personality. The Big Five Questionnaire was administered, consisting of adjectives as personality descriptors derived from lexical studies. When self-evaluating on adjectives that are, according to lexical hypothesis, the words that are most important for capturing the individual differences, and as such, the most frequent words in the vocabulary of many cultures, it is well possible that these words are good representatives of the well-behaved expressions, such as being "stable" or "flexible". As the other authors [24] also ar-

gue, it is possible that so-called higher-order factors (here secondary loaded factors) in fact represent moralistic bias rather than substantive dimensions of personality. Anyway, this finding is important since it shows that the lack of coercion and control by the therapist could stabilize a client in the well-behaved and socialized manner. Therapy Benefit contributing to plasticity, the latter, also referred to as dynamism or personal growth [23] was positively correlated with externalizing behavior (correlation $r = 0.75$) in other research [34], which denotes not only instability and lack of restraint, but also exploratory and approach-oriented behavior. Therefore, therapy benefit could have a direct effect on taking responsibility for own actions and incite client on more exploration in his/her life. Other important finding in this study was the better confirmation of the personality model with blended variables, instead of the higher-order factors. Another model including higher-order factors was also tested (but not reported in this study), and was outperformed by the blended-variables personality model. The result is in accordance with another study testing only the confirmation of the latent factors as the higher-order *vs* blended variables *vs* orthogonal factors [24], where the model presuming orthogonal factors was exceeded by the higher-order model, but the latter was exceeded by the blended variables model in three samples from Ontario, Oregon and Alberta. Other studies arguing for stability and plasticity as the higher-order factors do not provide unambiguous results for the theoretical regression of the factors onto higher-order factors (e.g. in a study [23] using also adjective personality markers, the two higher-order factors model fitted the data well, but openness/intellect did not load significantly on it). Such a result authors ascribe to the variation in markers descriptions. In our study of the blended variable model, where stability and plasticity are the blends of the Big Five factors, it is shown, that agreeableness, conscientiousness and neuroticism, besides loading on stability, substantially loaded on plasticity, as well. Neuroticism was better regressed onto plasticity ($r = -0.43$, $p < 0.01$), than stability, but this in fact may be due to plasticity feature encompassing some behaviors indicating instability. More studies in this domain considering personality models independently from exogenous variables, what this study actually has not specifically dealt with, could be useful.

This study has a few limitations that should be overcome in future research. Since this is the first study to bring therapist's styles into relation to personality, as well as to confirm the blended variable personality model, the study would be recommendable to replicate on a bit larger sample. Although the confirmation of personality model can be replicated in general population, therapist's styles assessment requires participants to be therapy-involved and that is why this study is unique in terms of the sample engaged. But further caution is advised when engaging clients involved in therapy process. What was not controlled for in this study and could have contributed to the ways questionnaires were fulfilled or the ways client perceived his/her therapist is the level of therapist's education. Some therapists in this study were able to do their own practice without supervision, while the others were in the process of doing the practice under supervision. In this research the level of therapist's education was not considered as the variable, but should be taken in regard in researches where therapists are in fact the subject of evaluation by client. This could even more objectify the evaluation independently given by client. Considering that psychotherapy in BH is still under intensive development, more researches that should follow the practice in counseling and psychotherapy should be welcomed. Another shortcoming is that we did not have pretest data for personality self-evaluation before the therapy process started, so it cannot be with certainty argued that therapy actually changed something in clients' personality traits. But this model gives general pattern as for the potential paths that should be given attention when demonstrating certain interpersonal styles. Correlational analyses showed significant results that did not hold in the direct paths of the model. This implies taking in account some other variables that were not included into this model. This research did not measure variables such as client wellbeing directly, but it would be interesting to examine whether personality or therapist's styles have better unique or common contribution to personal wellbeing. Instead of measuring personality as the disposition to behave, future studies should consider more concrete behaviors for the outcome variables. Personality inventories containing statements instead of one-word trait markers should be used in future research, since the latter can be more susceptible to self-presentation.

Conclusion

This study shows the underlying structure of therapist's styles could be best accounted for by the two preliminary extracted opposite styles labeled supportive autonomy and ignoring control, also confirmed in preliminary confirmatory analysis. Further analysis shows ignoring control and no supportive autonomy was correlated to personality self-evaluations on 5 personality traits. The more elaborate model gives further insight into the relationship, showing a few important relations: therapy benefit is predicted positively by supportive autonomy and negatively by ignoring control. Therapy benefit directly contributes to the account of plasticity, and ignoring control has a direct marginal effect on stability. Personality traits explained by stability also had secondary factor loadings on plasticity. Supportive autonomy has no direct or indirect effect either on stability or plasticity. This shows that Supportive autonomy is necessary but not enough condition for improvement in terms of manifesting more stable or exploring behavior. It is important to be careful when demonstrating directivity, especially if it leaves possibility to be blurred by control without support. A client's perception that the therapy is being helpful could instigate more explorative and approach-oriented behavior, what indirectly might contribute to client stability.

Acknowledgements

The authors would like to thank Luc Pelletier, Full Professor at School of Psychology, University of Ottawa for providing

us the original Therapist's Interpersonal Style Scale, as well as for his help in the double translation process.

The authors would also like to thank Saša Drače Associate Professor at the Department of Psychology, Faculty of Philosophy at the University of Sarajevo for providing important additional resources of scientific databases and Prof. Nazifa Savčić from English Department at the Faculty of Philosophy, University of Sarajevo for the help in back translation of the instrument.

The authors would like to thank the therapists who helped by recruiting their clients, as well as to the clients who participated in the study.

REFERENCES

1. *Ryan RM, Deci EL*. Self-determination theory and the facilitation of intrinsic motivation, social development, and well-being. Am Psychol 2000; 55(1): 68−78.
2. *Vansteenkiste M, Wiliams GC, Resnicow K*. Toward systematic integration between Self-Determination Theory and Motivational Interviewing as examples of top-down and bottom-up intervention development: Autonomy or volition as a fundamental theoretical principle. Int J Behav Nutr Phy 2012; 23(9): 1−11.
3. *Pelletier LG, Tuson KM, Haddad NK*. Client Motivation for Therapy Scale: a measure of intrinsic motivation, extrinsic motivation, and amotivation for therapy. J Pers Assess 1997; 68(2): 414−35.
4. *Dinger U, Strack M, Leichsenring F, Wilmers F, Schauenburg H*. Therapist effects on outcome and alliance in inpatient psychotherapy. J Clin Psychol 2008; 64(3): 344−54.
5. *Okiishi JC, Lambert MJ, Eggett D, Nielsen L, Dayton DD, Vermeersch DA*. An Analysis of Therapist Treatment Effects: Toward Providing Feedback to Individual Therapists on Their Clients'Psychotherapy Outcome. J Clin Psychol 2006; 62(9): 1157−72.
6. *Oliveira VC, Refsdhauge KM, Ferreira ML, Pinto RZ, Beckenkamp PR, Filho RF*, et al. Communication that values patient autonomy is associated with satisfaction with care: a systematic review. J Physiother 2012; 58(4): 215−29.
7. *Theodoridou A, Schlatter F, Ajdačić V, Rössler W, Jäger M*. Therapeutic relationship in the context of perceived coercion in a psychiatric population. Psychiatry Res 2012; 200(2−3): 939−44.
8. *Ackerman SJ, Hilsenroth MJ*. A review of therapist characteristics and techniques positively impacting the therapeutic alliance. Clin Psychol Rev 2003; 23(1): 1−33.
9. *Heinonen E, Lindfors O, Laaksonen MA, Knekt P*. Therapists' professional and personal characteristics as predictors of outcome in short- and long-term psychotherapy. J Affect Disord 2012; 138(3): 301−12.
10. *Watson JC*. Treatment failure in humanistic and experiential psychotherapy. J Clin Psychol 2011; 67(11): 1117−28.
11. *Henry WP, Schacht TE, Strupp HH, Butler SF, Binder JL*. Effects of training in time-limited dynamic psychotherapy: mediators of therapists' responses to training. J Consult Clin Psychol 1993; 61(3): 441−7.
12. *Ng CT, James S*. "Directive approach" for Chinese clients receiving psychotherapy: is that really a priority. Front Psychol 2013; 4(49): 1−13.
13. *Patrick H, Williams GC*. Self-determination theory: its application to health behavior and complementarity with motivational interviewing. Int J Behav Nutr Phy 2012; 9(18): 1−12.
14. *Swift JK, Callahan JL*. The impact of client treatment preferences on outcome: a meta-analysis. J Clin Psychol 2009; 65(4): 368−81.
15. *Mcmurtry SL, Hudson WW*. The Client Satisfaction Inventory. Results of an Initial Validation Study. Res Social Work Prac 2000; 10(5): 644−63.
16. *Landa AJ, Martos MP, Lopez-Zafra E*. Emotional intelligence and personality traits as predictors of psychological well-being in Spanish undergraduates. Soc Behav Personal 2010; 38(6): 783−94.
17. *Romero E, Gomez-Fraguela JA, Villar P*. Life aspirations, personality traits *and* subjective well-being *in a* Spanish sample. Eur J Personality 2012; 26(1): 45−55.
18. *Steel P, Schmidt J, Shultz J*. Refining the relationship between personality and subjective well-being. Psychol Bull 2008; 134(1): 138−61.
19. *Lucas RE, Diener E*. Personality and subjective well-being. In: *John OP, Robins R, Pervin LA*. , editors. Handbook of personality : theory and research. 3rd ed. New York: Guilford; 2008. p. 795−814.
20. *Weiss A, Bates TC, Luciano M*. Happiness is a personal(ity) thing: the genetics of personality and well-being in a representative sample. Psychol Sci 2008; 19(3): 205−10.
21. *Gutiérrez JL, Jiménez BM, Hernández EG, Puente CP*. Personality and subjective well-being: big five correlates and demographic variables. Pers Indiv Differ 2005; 38: 1561−9.
22. *Gale CR, Booth T, Mõttus R, Kuh D, Deary IJ*. Neuroticism and Extraversion in Youth Predict Mental Wellbeing and Life Satisfaction 40 Years Later. J Res Pers 2013; 47(6): 687−97.
23. *DeYoung CG*. Higher-order factors of the Big Five in a multi-informant sample. J Pers Soc Psychol 2006; 91(6): 1138−51.
24. *Ashton MC, Lee K, Goldberg LR, de Vries RE*. Higher order factors of personality: do they exist. Pers Soc Psychol Rev 2009; 13(2): 79−91.
25. *del Re AC, Flückiger C, Horvath AO, Symonds D, Wampold BE*. Therapist effects in the therapeutic alliance-outcome relationship: a restricted-maximum likelihood meta-analysis. Clin Psychol Rev 2012; 32(7): 642−9.
26. *Pelletier LG, Fortier MS, Vallerand RJ, Tuson KM, Brière NM, Blais MR*. Toward a New Measure of Intrinsic Motivation, Extrinsic Motivation, and Amotivation in Sports: The Sport Motivation Scale (SMS). J Sport Exerc Psychol 1995; 17: 35−53.
27. *Kardum I, Smojver I*. Five Factor Model of Personality Structure: Item Selection descriptors in Croatian language. Godišnjak Zavoda za psihologiju 1993; 2(1): 91−100. (Croatian)
28. *Field A*. Discovering Statistics Using SPSS. 3rd. London: Sage Publications; 2009.
29. *Hair FH, Black WC, Babin BJ, Anderson RE*. Multivariate Data Analysis. 7th ed. New Jersey (US): Pearson Prentice Hall; 2010.
30. *Jöreskog KG, Sörbom D*. LISREL-VI user's guide. 3rd ed. Mooresville (IN): Scientific Software; 1984.
31. *Byrne BM*. Structural Equation Modeling with AMOS. 2nd ed. New York (US): Routledge, Taylor and Francis Group; 2010.
32. *Browne MW, Cudeck R*. Alternative ways of assessing model fit. In: *Bollen KA, Long JS*, editors. Testing Structural Equation Models. Newbury Park: Sage 1993. p. 136−62.
33. *Bentler PM, Bonett DG*. Significance tests and goodness of fit in the analysis of covariance structures. Psychol Bull 1980; 88(3): 588−606.
34. *de Young CG, Peterson JB, Séguin JR, Tremblay RE*. Externalizing behavior and the higher order factors of the Big Five. J Abnorm Psychol 2008; 117(4): 947−53.

Received on September 11, 2014.
Revised on November 10, 2014.
Accepted on December 4, 2014.
Online First December, 2015.

Appendix

The Adapted Therapist's Interpersonal Style Scale (ATIS)

1----------2----------3----------4----------5----------6----------7
Never Often Always

1. The feedback I receive from my therapist is constructive in helping me make improvements.
2. When I ask my therapist to help me solve a problem, he or she asks me what I think before giving me his or her opinion.
3. My therapist gives me the feedback about the way I make a progress.
4. My therapist is trying to impose her/his ideas on me.
5. The feedback I get from my therapist is basically useless criticism.
6. I feel that my therapist is indifferent towards me.
7. My therapist pressures me to do what he or she wants.
8. My therapist consults me before (s)he decides how to address my problems.
9. My therapist is being harsh to me.
10. I feel that my therapist doesn't care how much I improve through therapy.
11. My therapist provides me with lots of opportunities to take personal decisions in what I do.
12. My therapist leaves me with little choice about the ways in which my problems could be resolved.

ORIGINAL ARTICLE

UDC: 615.03:[615.099:615.2/.3
DOI: 10.2298/VSP141222019D

Determination of flumazenil in serum by liquid chromatography-mass spectrometry: Application to kinetics study in acute diazepam overdose

Određivanje flumazenila u serumu tečnom hromatografijom sa masenom spektrometrijom: primena u kinetičkoj studiji kod akutnog predoziranja diazepamom

Snežana Djordjević*†, Jasmina Jović-Stošić*†, Vesna Kilibarda*†, Zoran Šegrt†, Nataša Perković-Vukčević*†

***National Poison Control Center, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Backgound/Aim. Flumazenil is benzodiazepine receptor antagonist. It has been studied for a various indications, including reversal of sedation after surgery or diagnostic procedures, awakening of comatose patients in benzodiazepine overdose, or for symptomatic treatment of hepatic encephalopathy. Some drugs, like theophylline, may prolong its elimination half-life. Considering the long half-life of diazepam and its metabolites, concomitant use of theophylline may reduce the need for repeated dosing of flumazenil in patients with acute diazepam poisoning. The aim of this study was to introduce a reliable and accurate method for determining the concentration of flumazenil after therapeutic application in patients with acute poisoning, and using that method to assess whether the kinetics of flumazenil change in the presence of aminophylline (combination of theophylline and ethylenediamine in a 2 : 1 ratio) applied as concomitant therapy. **Methods.** Blood samples from patients with acute diazepam poisoning that received flumazenil at the dose of 0.5 mg, or the same dose with 3 mg/kg of body weight of aminophylline, were collected 1, 3, 10, 30, 60, 120 and 240 min after its intravenous administration. Samples were prepared by solid-phase extraction on Oasis HLB cartridges with ethylacetate as extracting agens. Flumazenil was determined by liquid chromatography with mass spectrometry (LC-MS) in single ion monitoring mode at m/z 304. Separation of flumazenil from matrix compound was performed on Lichrospher RP-8 column using the mixture of acidic acetonitrile and 20 mM of ammonium acetate in water (55 : 45) as a mobile phase. **Results.** The applied analitycal method showed excellent recovery (94.65%). The obtained extracts were much cleaner than the extracts obtained by the same extractant in the process of liquid-liquid extraction. The limit of detection of the LC-MS method described in this paper was 0.5 ng/mL and the limit of quantitation was 1 ng/mL. In the patients treated with both flumazenil and aminophylline, the elimination constant for flumazenil was significantly lower and the elimination half-life was longer ($p < 0.05$) in comparison with the same parameters in the patients who received flumazenil alone. **Conclusion.** The applied LC-MS method for the determination of flumazenil in serum samples of patients with acute diazepam poisoning is rapid, sensitive, precise and specific. Concomitant use with theophylline significantly prolonged elimination of flumazenil during the treatment of acute poisonings with diazepam.

Key words:
diazepam; poisoning; flumazenil; aminophylline; chromatography, liquid; mass spectrometry.

Apstrakt

Uvod/Cilj. Flumazenil je antagonist benzodiazepinskih receptora čiji su efekti ispitivani kod različitih indikacija kao što su reverzija sedacije posle hirurških intervencija ili dijagnostičkih procedura, terapija kome u akutnim trovanjima ili simptomatska terapija hepatične encefalopatije. Pojedini lekovi, kao što je teofilin, mogu dovesti do produženja poluvremena eliminacije flumazenila. Imajući u vidu dugo poluvreme eliminacije diazepama i njegovih metabolita, istovremena upotreba teofilina sa flumazenilom bi smanjila potrebu za ponovljenim davanjem flumazenila kod bolesnika sa akutnim trovanjem diazepamom. Stoga, cilj ovog rada bio je uvođenje pouzdane i precizne metode za određivanje koncentracije flumazenila u krvi nakon terapijske primene kod bolesnika sa akutnim trovanjem, a zatim, primenom ove metode utvrđivanje da li dolazi do izmena u kinetici flumazenila u prisustvu istovremeno primenjivanog aminofilina (kombinacija teofilina i etilendiamina u odnosu 2 : 1). **Metode.** Uzorci krvi bolesnika sa akutnim trovanjem diazepamom koji su dobili samo flumazenil u dozi od 0,5 mg ili istovremeno sa 3 mg/kg aminofilina, uzeti su 1, 3, 10, 30, 60, 120 i 240 min nakon njegove intravenske primene. Uzorci su pripremani čvrsto-faznom ekstrakcijom

Correspondence to: Snežana Djordjević, National Poison Control Center, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. E-mail: ivezicnela@yahoo.com

(SPE) na Oasis HLB kertridžima sa etilacetatom kao ekstrakcionim agensom. Flumazenil je određen tečnom hromatografijom sa masenom spektrometrijom (LC-MS) u *single ion monitoring* (SIM) modu na m/z 304. Razdvajanje flumazenila od komponenti matriksa izvršeno je na Lichrospher RP-8 koloni uz korišćenje smeše kiselog acetonitrila i 20 mM amonijum acetata u vodi (55 : 45) kao mobilne faze. **Rezultati.** Primenjena analitička metoda pokazala je odličan analitički prinos (94.65%). Dobijeni ekstrakti bili su čistiji nego ekstrakti dobijeni pomoću istog ekstraktanta nakon tečno-tečne ekstrakcije. Limit detekcije i limit kvantifikacije (LoQ) opisane LC-MS metode bili su 0,5 ng /mL i 1 ng/mL. Kod bolesnika lečenih istovremenom primenom flumazenila i aminofilina, konstanta eliminacije za flumazenil bila je značajno veća, a poluvreme eliminacije značajno duže u odnosu na ove parametre praćene u grupi bolesnika koja je primila samo flumazenil ($p < 0,05$). **Zaključak.** Primenjena LC-MS metoda za određivanje flumazenila u serumu bolesnika sa akutnim trovanjem diazepamom je brza, osetljiva, precizna i specifična. Istovremena primena teofilina značajno produžava eliminaciju flumazenila prilikom lečenja akutnih trovanja diazepamom.

Ključne reči:
diazepam; trovanje; flumazenil; aminofilin; hromatografija, tečna; spektrometrija mase.

Introduction

Flumazenil, an imidazobenzodiazepine, is a competitive antagonist of benzodiazepine receptors. It selectively binds to these receptors in the central nervous system, thus blocking activation of inhibitory gama-aminobutyric acid (GABA)-ergic synapses. This way, flumazenil antagonizes central effects of substances which manifest their activity through benzodiazepine receptors [1–3]. Flumazenil has been studied for a various indications, including reversal of sedation after short-lasting surgery or diagnostic procedures like endoscopy, awakening of comatose patients in benzodiazepine overdose, or for symptomatic treatment of hepatic encephalopathy [4–7].

Flumazenil may be administered as an antidote in acute poisoning with benzodiazepines [8, 9], but it should not be used in patients with the history of epilepsy or with benzodiazepine intoxication combined with tricyclic antidepressants [10, 11]. Flumazenil may precipitate withdrawal syndrome, cardiovascular effects, or seizures in overdosed benzodiazepine dependent patients [8, 12, 13]. Because of contraindications and adverse effects, flumazenil must be used with caution in poisoning with benzodiazepines. Although it increases the level of consciousness in benzodiazepines poisonings, because many benzodiazepines have a longer half-life than flumazenil, resedation is possible soon after application, and therefore, sometimes it is necessary to apply several doses of the drug to improve the therapeutic efficiency [8].

Flumazenil does not alter the pharmacokinetics of benzodiazepines [14], end the extent to which flumazenil antagonizes effects of benzodiazepines depends on the dose and the concentration of both drugs in plasma [1]. The metabolism of flumazenil is rapid and extensive, and takes place in the liver. The medium half-life of flumazenil in plasma is about 54 min (41–79 min) [1], but there are some substances, like theophylline which could prolong its half-life [15].

Determination of flumazenil in serum samples may be carried out using various chromatographic techniques [15–27]. Often it has been applied to high performance liquid chromatography (HPLC) with ultraviolet detection (HPLC-UV) [16–21], but a more specific and sensitive method is liquid chromatography with mass spectrometry (LC-MS) detection [22–25].

Thus, the aim of this study was to introduce a reliable and accurate method for determining the concentration of flumazenil after therapeutic application in patients with acute diazepam poisoning, and using that method to assess whether the kinetics of flumazenil change in the presence of aminophylline combination that contains teophylline and ethylenediamine in a 2 : 1 ratio) applied as parallel therapy, because slowing of elimination may prolong its antidotal action and thus reduce the need for repeated doses.

Methods

Material

Flumazenil and fluoxetine (an internal standard) analytical standards were obtained from the companies Roche (Basel, Switzerland) and Sigma-Aldrich (St. Luis, Missouri, United States), respectively. HPLC grade acetonitrile and methanol, as well as acetic acid, ammonium acetate, ethyl acetate and hydrochloric acid p.a, were obtained from Merck (Darmstadt, Germany). Water was purified by Millipore Milli-Q system. Cartridges for solid-phase extraction Oasis HLB 30 μm, 1 mL, were obtained from Waters (Manchester, United Kingdom).

Blood samples from the two groups of patients (10 persons each) with acute diazepam poisoning, who received flumazenil at the dose of 0.5 mg, or the same dose with the 3 mg/kg of body weight of aminophylline, were collected 1, 3, 10, 30, 60, 120 and 240 min after intravenous administration.

Method

For determination of flumazenil in serum a mass spectrometer with chemical ionization at atmospheric pressure (Finnigan MAT SSQ7000 LC/MS – ESI System) with HPLC P2000 binary pump, degasser SCM1000 and autosampler AS3000 were used. Mobile phase was a mixture of the solution A (acetonitrile: glacial acetic acid = 99 : 1) and B (20 mM of ammonium acetate in water) in the ratio of 55 : 45. The flow rate of mobile phase was 1 mL/min. Separation of flumazenil and internal standard from matrix compound was performed on a column Lichrospher 100 RP-8 E 250-4, 5 μm (Merck), with guard column Lichrochart 4–4 RP-8 (Merck) at ambient temperature after injection of 50 μL of sample.

A mass detector was adjusted to work in a single ion monitoring (SIM) mode for masses m/z 304 and 310 for flumazenil and internal standard, respectively. The electron multiplier voltages was 2,200 V. The capillary and the tube lens voltages were

26.8 V and 115.9 V, respectively. The pressure of the main and the auxiliary gas (N2) was 60 and 150 psi, respectively.

Preparation of a standard solution and samples

The stock standard solution of flumazenil was prepared by dissolving 10 mg in 10 mL acetonitrile and stored at +4ºC. Calibration curve solutions were prepared by adding flumazenil standard solution in pool serum and prepared like serum samples.

Extraction of flumazenil from serum samples was performed on the Oasis HLB cartridge, previously activated with 1 mL of methanol and 1 mL demineralised water. In a serum sample 0.05 mL of internal standard (fluoxetine) and 0.1 mL 1M hydrochloric acid were added. After mixing and centrifugation at 8,360 rpm, a sample was loaded to the activated cartridge. The cartridge was washed with 1 mL of 5% methanol. Elution of flumazenil and the internal standard (IS) is carried out with 3 mL of ethyl acetate. The obtained eluate was evaporated under the stream of air to dryness, reconstituted in 1 mL of mobile phase and analyzed by the LC-MS method.

Comparison of the mean flumazenil maximum concentration (Cmax), elimination constant (Ke) and elimination half-life ($t_{1/2}$) after its applying alone or in combination with aminophylline was done by Student`s *t*-test.

Results

Using the described method, retention times for flumazenil and internal standard were 4.4 min and 2.5 min, respectively. Figure 1 shows the mass spectrum of flumazenil.

Calibration curve solutions were prepared by adding a flumazenil standard solution in pool serum and prepared like serum samples. The calibration curve was linear in the concentrations range of 1; 2.5; 5; 10; 25; 50 and 100 ng/mL (Figure 2).

Chromatograms of the internal standard, serum spiked with

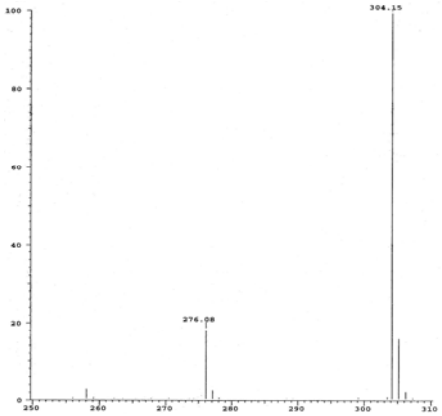


Fig. 1 – Mass spectrum of flumazenil.

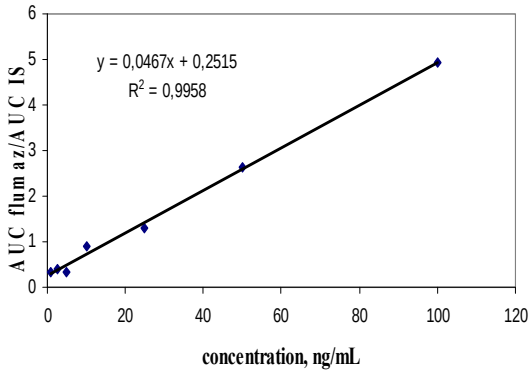
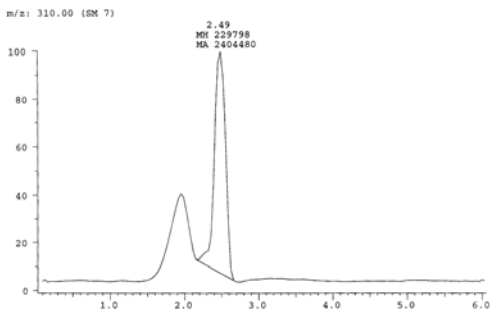
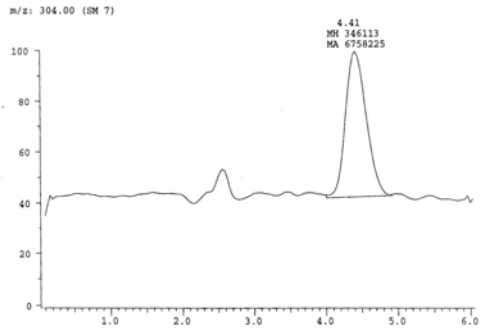


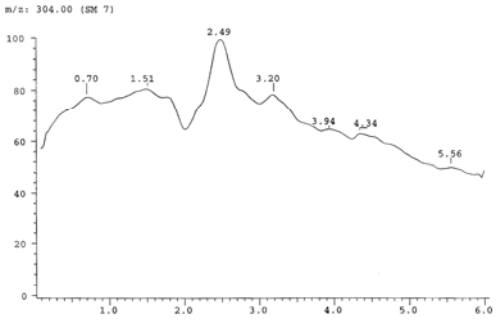
Fig. 2 – Calibration curve of serum spiked with flumazenil. AUC – area under the curve; IS – internal standard



a)



b)



c)

Fig. 3 – Chromatogram of: a) internal standard, b) serum spiked with flumazenil and c) pool serum.

100 ng/mL of flumazenil and pool serum are shown in Figure 3.

The intra-day precision of the method was assessed by calculating the coefficient of variation (CV) for the measured parameter of the method (ratio of peak area of flumazenil and IS) and determined on the same day. It was done by preparing ten flumazenil standard samples with concentration of 10 ng/mL and determining by the LC-MS method. The CV was 5.18%. The inter-day CVs for spiked serum were also acceptable and are shown in Table 1.

The mean analytical recovery was 94.65% (ranged from 91.48 to 99.13%). Table 2 shows the analytically recovery from the serum after solid-phase extraction with ethyl acetate on the Oasis HLB cartridges.

The limit of detection (LoD) was defined as the concentration at which the signal to noise ratio is equal to, or greater than three, and the limit of quantitation (LoQ) was defined as the concentration at which the signal to noise ratio is equal to, or greater than ten. Accordingly, LoD and LoQ were 0.5 ng/L and 1.0 ng/mL, respectively.

Determination of flumazenil in serum samples of patients was carried out on the basis of the equation of the calibration curve, which was obtained upon the analysis of spiked serum. Linear regression of flumazenil was y = 0.0467 x + 0.2515 (R = 0.9958 for the concentration range of 1 to 100 ng/mL). Main pharmacokinetic parameters of flumazenil including Cmax, Ke and $t_{1/2}$ are listed in Table 3. Student's t-test revealed a significantly lower Ke ($p < 0.05$) and a significantly longer $t_{1/2}$ ($p < 0.05$) in patients treated with both flumazenil and aminophylline.

Table 1

Inter-day coefficient of variation (CV) for determination of flumazenil by liquid chromatography with mass spectrometry (LC-MS) method

Added concentration (mg/L)	1st day			2nd day		
	Obtained concentration (mg/L)	x̄ ± SD	CV	Obtained concentration (mg/L)	x̄ ± SD	CV
1.0	10.15 10.97 9.37	10.16 ± 0.79	7.86%	10.14 8.55 10.09	9.59 ± 0.90	9.42%
10.0	49.23 48.66 52.53	50.14 ± 2.09	4.16%	49.432 48.11 51.98	49.84 ± 1.96	3.94%
100.0	101.23 103.42 98.01	100.88 ± 2.72	2.70%	100.98 102,90 97,16	100.35 ± 2.92	2.91%

x̄ – mean value; SD – standard deviation; CV – coefficent of veriation.

Table 2

Analytical recovery for flumazenil

Flumazenil conc. (ng/mL)	AUC flumazenil/ AUC IS		Recovery (%)
	Standard	Spiked serum	
1	0.3507	0.3208	91.48
2.5	0.4195	0.3909	93.18
5	0.3615	0.3437	95.07
10	0.9528	0.8994	94.39
25	1.3942	1.2920	92.67
50	2.6574	2.6342	99.13
100	5.0906	4.9211	96.67

AUC – arca under the curve; IS – internal standard.

Table 3

Pharmacokinetic parameters of flumazenil in patients treated with flumazenil only or with flumazenil and aminophylline

Patient	flumazenil			flumazenil + aminophylline		
	C_{max} (ng/mL)	Ke (min^{-1})	$t_{1/2}$ (min)	C_{max} (ng/mL)	Ke (min^{-1})	$t_{1/2}$ (min)
1.	34.65	0.0211	32.87	22.78	0.0156	44.46
2.	28.72	0.0168	41.22	92.60	0.0092	75.40
3.	94.33	0.0093	74.62	81.02	0.0050	137.63
4.	76.91	0.0131	52.67	131.87	0.0070	98.84
5.	26.45	0.0100	69.01	28.30	0.0050	137.42
6.	19.82	0.0121	57.29	27.53	0.0075	91.86
7.	41.92	0.0127	54.33	60.56	0.0071	98.07
8.	65.34	0.0091	76.35	21.07	0.0037	188.25
9.	107.27	0.0171	40.46	43.62	0.0097	71.18
10.	23.46	0.0115	60.36	15.42	0.0048	144.96
x̄ ± SD	51.89±31.78	0.0133±0.0039	55.92±14.75	52.48±38.47	0.0075±0.0035	108.81±42.47

C_{max} – maximum concentration; Ke – elimination constant; $t_{1/2}$ – elimination half-life; x̄ – mean value; SD – standard deviation.

Discussion

Isolation of flumazenil from the biological material can be done by liquid-liquid [25] or solid-phase extraction [20]. Previously reported analytical recovery for flumazenil after solid-phase extraction was 78% [21]. In our study, the applied extraction on Oasis HLB cartridges with ethyl acetate as extractant, for preparation of serum samples from acutely poisoned patients showed better recovery (94.65%). Uppon our previous experience in flumazenil determination, the obtained extracts were much cleaner than the extracts obtained by the same extractant in the process of liquid-liquid extraction (data not shown). We found that in comparisson with liquid-liquid extraction, solid-phase exraction is simpler, faster to perform and safer for analyst, which is of great importance when it is necessary to analyze a large number of samples.

The literature describes a variety of chromatographic techniques for the determination of flumazenil such as gas chromatography with nitrogen-phosphorus or mass spectrometric detectors and HPLC-UV, LC-MS [15–27]. Thus, Bun et al. [17] described the HPLC-UV method for determination of flumazenil in serum at 245 nm with the detection limit of 2 ng/mL. Similar result was obtained by Zedkova et al. [18], with the detection limit of 2.5 ng/mL and detection at 250 nm.

Liquid chromatography coupled with mass-spectrometric detection is the most sensitive and the most specific analytical method of drugs in biological samples. Generally, the sensitivity of this method may be increased performing tandem mass spectrometry (LC-MS-MS). The reported limit of detection using LC-ESI-MS method for flumazenil was 2.5 ng/mL[21]. However, the limit of detection of the LC-MS method described in this paper was lower and achieved 0.5 ng/mL, while the limit of quantification was 1 ng /mL.

We applied the described LC-MS method for determination of flumazenil in serum samples and used the obtained data for calculating the pharmacokinetic parameters: c_{max}, $t_{1/2}$ and Ke. According to the literature, the mean c_{max} of flumazenil in plasma after intravenous infusion of 2 mg of this drug was 55 ng/mL [1].

Our data on the flumazenil concentration in serum of patients poisoned by diazepam showed significant inter-individual differences, which are in accordance with the fact that the drug is administered in a fixed dose to patients with different pharmacokinetic properties.

The mean $t_{1/2}$ of flumazenil in the group of patients overdosed with diazepam receiving the drug was 55.92 ± 14.75 which is similar to literature data of 54 min [1]. However, in the group of patients receiving both flumazenil and aminophylline, the mean $t_{1/2}$ of flumazenil was longer (almost double) (108.81 ± 42.47 min). The Ke of flumazenil in this group was also lower (0.0075 ± 0.0035 min^{-1}) than in the group receving only flumazenil (0.0133 ± 0.0039 min^{-1}). Despite the great interindividual variance, the results for the Ke and $t_{1/2}$ of flumazenil showed a statistically significant slowing of flumazenil elimination in the presence of theophylline in blood.

The results of limited previously published studies showed that combined application of flumazenil and theophylline resulted in a prolonged $t_{1/2}$ of flumazenil in rabbits [28]. Also, in patients sedated with midazolam, Bonfiglio et al. [15] revealed that theophylline appeared to significantly prolong the half-life of flumazenil. However, the mechanism of interaction of these two drugs is not known.

The main metabolic transformation of flumazenil involves the activation of carboxylesterase to form flumazenil acid as the major metabolite which is without pharmacological activity. In a small percentage flumazenil may be demehylated through cytochrome P450. The metabolism of theophylline involves mainly hydroxylation and demethylation. In both processes cytochrome P450 oxidase is involved [29,30]. This fact supports the hypothesis that in the case of combined use, flumazenil and theophylline may compete for binding to the same enzyme involved in the process of demethylation.

In recent years, effects of flumazenil and aminophylline have been investigated in reversal of different kinds of anesthesia [31–34]. Concomitant use of both drugs may also be explored, having in mind their synergic action and interactions. Extended half-life of flumazenil in combination with theophylline may also be of importance in the treatment of poisonings with long-acting benzodiazepines.

Conclusion

The applied liquid chromatography with mass spectrometry method for the determination of flumazenil in serum samples of patients acutely poisoned with diazepam is rapid, sensitive, precise and specific. The applied solid-phase extraction gave very good recovery, which is very important considering low concentrations in samples. The method is applicable to the routine determination of flumazenil serum concentrations, as well as in pharmacokinetic studies. Also, our results confirm previous findings that the concomitant use of theophylline significantly prolongs elimination of flumazenil during the treatment of acute poisonings with diazepam.

REFERENCES

1. Anexate (Flumazenil) product monograph. Basle, Switzerland: F. Hoffmann-La Roche & Co. Limited Company; 1987. [published 2008 December 30]. Available from: http://rochecanada.com/fmfiles/re7234008/Research/ClinicalTrialsForms/Products/Consumer information/ Monographsand-Public Advisories/Anexate/anexateJune3pmE.pdf.
2. *Votey SR, Bosse GM, Bayer MJ, Hoffman JR.* Flumazenil: a new benzodiazepine antagonist. Ann Emerg Med 1991; 20(2): 181–8.
3. *Whitwam JG, Amrein R.* Pharmacology of flumazenil. Acta Anaesthesiol Scand Suppl 1995; 108: 3–14.
4. *Wille RT, Chaffee BW, Ryan ML, Elta GH, Walter V, Barnett JL.* Pharmacoeconomic evaluation of flumazenil for routine outpatient EGD. Gastrointest Endosc 2000; 51(3): 282–7.
5. *Krisanda TJ.* Flumazenil: an antidote for benzodiazepine toxicity. Am Fam Physician 1993; 47(4): 891–5.

6. *Weinbroum A, Rudick V, Sorkine P, Nevo Y, Halpern P, Geller E,* et al. Use of flumazenil in the treatment of drug overdose: a double-blind and open clinical study in 110 patients. Crit Care Med 1996; 24(2): 199−206.
7. *Als-Nielsen B, Kjaergard LL, Gluud C.* Benzodiazepine receptor antagonists for acute and chronic hepatic encephalopathy. Cochrane Database Syst Rev 2001; (4): CD002798.
8. *Hoffman EJ, Warren EW.* Flumazenil: a benzodiazepine antagonist. Clin Pharm 1993; 12(9): 641−56.
9. *Mégarbane B, Buisine A, Jacobs F, Résière D, Chevillard L, Vicaut E,* et al. Prospective comparative assessment of buprenorphine overdose with heroin and methadone: clinical characteristics and response to antidotal treatment. J Subst Abuse Treat 2010; 38(4): 403−7.
10. *Woolf AD, Erdman AR, Nelson LS, Caravati E, Cobaugh DJ, Booze LL,* et al. Tricyclic antidepressant poisoning: an evidence-based consensus guideline for out-of-hospital management. Clin Toxicol (Phila) 2007; 45(3): 203−33.
11. *Lopez A, Rebollo J.* Benzodiazepine withdrawal syndrome after a benzodiazepine antagonist. Crit Care Med 1990; 18(12): 1480−1.
12. *Marchant B, Wray R, Leach A, Nama M.* Flumazenil causing convulsions and ventricular tachycardia. BMJ 1989; 299(6703): 860.
13. *Thomson JS, Donald C, Lewin K.* Use of Flumazenil in Benzodiazepine overdose. Emerg Med J 2006; 23(2): 162.
14. *Klotz U, Duka T, Dorow R, Doenicke A.* Flunitrazepam and lormetazepam do not affect the pharmacokinetics of the benzodiazepine antagonist Ro 15-1788. Br J Clin Pharmacol 1985; 19(1): 95−8.
15. *Bonfiglio MF, Fisher-Katz LE, Saltis LM, Traeger SM, Martin BR, Nackes NA,* et al. A pilot pharmacokinetic-pharmacodynamic study of benzodiazepine antagonism by flumazenil and aminophylline. Pharmacotherapy 1996; 16(6): 1166−72.
16. *Scheepers LD, Montgomery CJ, Kinahan AM, Dunn GS, Bourne RA, McCormack JP.* Plasma concentration of flumazenil following intranasal administration in children. Can J Anaesth 2000; 47(2): 120−4.
17. *Bun H, Duplan V, Crevat-Pisano P, Llurens M, Durand A.* Rapid determination of the benzodiazepine antagonist flumazenil, Ro 15-1788, by high performance liquid chromatography. Biomed Chromatogr 1989; 3(6): 269−71.
18. *Zedkova L, Rauw GA, Baker GB, Coupland NJ.* A rapid high-pressure liquid chromatographic procedure for determination of flumazenil in plasma. J Pharmacol Toxicol Methods 2001; 46(1): 57−60.
19. *Vletter AA, Burm AG, Breimer LT, Spierdijk J.* High-performance liquid chromatographic assay to determine midazolam and flumazenil simultaneously in human plasma. J Chromatogr 1990; 530(1): 177−85.
20. *Chan K, Jones RD.* Simultaneous determination of flumazenil, midazolam and metabolites in human biological fluids by liquid chromatography. J Chromatogr 1993; 619(1): 154−60.
21. *Lavén M, Appel L, Moulder R, Tyrefors N, Markides K, Långström B.* Determination of flumazenil in human plasma by liquid chromatography-electrospray ionisation tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 808(2): 221−7.
22. *Kanazawa H, Nagata Y, Matsushima Y, Takai N, Uchiyama H, Nishimura R,* et al. Liquid chromatography-mass spectrometry for the determination of medetomidine and other anaesthetics in plasma. J Chromatogr 1993; 631(1−2): 215−20.
23. *Lavén M, Markides K, Långström B.* Analysis of microsomal metabolic stability using high-flow-rate extraction coupled to capillary liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 806(2): 119−26.
24. *Djordjevic S, Kovacevic I, Miljkovic B, Vuksanovic J, Pokrajac M.* Liquid chromatographic-mass spectrometric method for the determination of fluoxetine and norfluoxetine in human plasma: application to clinical study. Farmaco 2005; 60(4): 345−9.
25. *Kratzsch C, Tenberken O, Peters FT, Weber AA, Kraemer T, Maurer HH.* Screening, library-assisted identification and validated quantification of 23 benzodiazepines, flumazenil, zaleplone, zolpidem and zopiclone in plasma by liquid chromatography/mass spectrometry with atmospheric pressure chemical ionization. J Mass Spectrom 2004; 39(8): 856−72.
26. *Song D, Khaykis V, Kohlhof K.* Determination of flumazenil in plasma by gas chromatography-negative ion chemical ionization mass spectrometry. J Chromatogr B Biomed Appl 1995; 663(2): 263−73.
27. *Fisher LE, Perch S, Bonfiglio MF, Geers SM.* Simultaneous determination of midazolam and flumazenil concentrations in human plasma by gas chromatography. J Chromatogr B, Biomed Appl 1995; 665(1): 217−21.
28. *Najjar TA, Al-Hassan MI, Khan RM.* Theophylline inhibits the elimination of flumazenil in rabbits. Int J Pharm 1993; 98(1−3): 51−5.
29. *Klotz U, Kanto J.* Pharmacokinetics and Clinical Use of Flumazenil (Ro 15-1788). Clin Pharmacokin 1988; 14(1): 1−12.
30. *Kleingeist B, Bocker R, Geisslinger G, Brugge R.* Isolation and pharmacological characterization of microsomal human liver flumazenil carboxylesterase. J Pharm Pharmaceut Sci 1998; 1(1): 38−46.
31. *Dahaba AA, Bornemann H, Rehak PH, Wang G, Wu XM, Metzler H.* Effect of flumazenil on bispectral index monitoring in unpremedicated patients. Anesthesiology 2009; 110(5): 1036−40.
32. *Kim YJ, Lee H, Kim CH, Lee GY, Baik HJ, Han JI.* Effect of flumazenil on recovery from anesthesia and the bispectral index after sevoflurane/fentanyl general anesthesia in unpremedicated patients. Korean J Anesthesiol 2012; 62(1): 19−23.
33. *Turan A, Memiş D, Karamanlıoğlu B, Pamukçu Z, Süt N.* Effect of aminophylline on bispectral index. Acta Anaesthesiol Scand 2004; 48(4): 408−11.
34. *Kim DW, Joo JD, In JH, Jeon YS, Jung HS, Jeon KB, Choi JW.* Comparison of the recovery and respiratory effects of aminophylline and doxapram following total intravenous anesthesia with propofol and remifentanil. J Clin Anesth 2013;25(3):173−6.

Received on December 22, 2014.
Revised on January 25, 2015.
Accepted on February 27, 2015.
Online First April, 2015.

ORIGINAL ARTICLE

UDC: 340.6:616-091.5-074
DOI: 10.2298/VSP141111026S

Association between ambient air pollution, meteorological conditions and exacerbations of asthma and chronic obstructive pulmonary disease in adult citizens of the town of Smederevo

Povezanost aerozagađenja i meteoroloških uslova sa pogoršanjima astme i hronične opstruktivne bolesti pluća kod odraslih stanovnika Smedereva

Ivan Stevanović*, Milena Jovašević-Stojanović†, Jasmina Jović Stošić‡§

***Department of Internal Medicine, General Hospital Smederevo, Smederevo, Serbia; †Vinča Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia; ‡National Poison Control Center, Military Medical Academy, Belgrade, Serbia; §Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Introduction. Smederevo is the only town in Serbia with a steel factory, whose exhausts contribute to air pollution. Therefore, the city conducts continuous monitoring of air quality. In recent years, high levels of particulate matter (PM), including coarse (PM_{10}) and fine ($PM_{2.5}$) particles in the air have frequently been recorded. The aim of this study was to assess association between exacerbation of asthma or chronic obstructive pulmonary disease (COPD) in adults and air pollution or meteorological conditions. **Methods.** The study was conducted in the secondary care General Hospital in Smederevo covering approximately 81, 000 inhabitants living in the area of about 7 km around the automatic station for air quality monitoring from which the verified data were collected. Data on patients were obtained from medical records. The correlation between the incidence of diseases exacerbation and the number of days with exceedance of air pollutants limit level *per* month, as well as meteorological conditions, was tested with parametric Pearson bivariate correlation test in program SPSS. **Results.** The study population consisted of adults registered as asthma or COPD suffering patients (n = 1,624) with 570 episodes of remarkable exacerbations (moderate or severe) of the disease in 2011. Asthma exacerbation was significantly more frequent in women than in men. The number of days with high levels of $PM_{2.5}$ *per* month was statistically significantly associated with the total number of exacerbation (moderate and severe of both asthma and COPD) episodes among the female patients. There was also a statistically significant association between the number of days with $PM_{2.5}$ exceedance and the number of moderate exacerbations in the subgroups of non-smokers and obese patients. A significant correlation of the number of days with the exceedance of PM_{10} limit level was shown only for the subgroup of obese, non-smoking patients with moderate exacerbation. A significant negative association with the average ambient temperature was proven for the obese female patients and obese non-smoking patients with moderate asthma exacerbations. The number of COPD exacerbation was in positive correlation with the average air pressure for the subgroup of female smokers, but the connection with air pollution was not proven. **Conclusion.** Exposure to airborne particles in the town of Smederevo, mainly to $PM_{2.5}$, and to low temperature may trigger asthma exacerbation requiring emergency care. The most vulnerable may be women and obese patients.

Key words:
air pollution; particle size; weather; disease progression; risk factors; pulmonary disease, chronic obstructive; asthma.

Apstrakt

Uvod/Cilj. Smederevo je jedini grad u Srbiji u kome postoji železara čiji rad doprinosi aerozagađenju. Stoga se kontinuirano kontroliše kvalitet vazduha. Poslednjih godina beleže se visoke koncentracije suspendovanih čestica (*paticulate matter* – PM), grubih čestica PM_{10} i finih čestica ($PM_{2.5}$). Cilj rada bio je da se ispita da li postoji povezanost pogoršanja respiratornih oboljenja, hronične opstruktivne bolesti pluća (HOBP) i astme, sa aerozagađenjem i meteorološkim faktorima. **Metode.** Ispitivanje je obavljeno u Opštoj bolnici u Smederevu koja pokriva oko 81 000 stanovnika, nastanjenih u krugu od približno 7 kilometara oko automatske stanice za praćenje kvaliteta vazduha, sa koje su dobijeni verifikovani podaci o nivou aerozagađivača i o meteorološkim faktorima. Podaci o bolesnicima su dobijeni iz medicinske dokumentacije. Korelacija učestalosti pogoršanja bolesti sa brojem dana u mesecu tokom kojih je zabeleženo prekoračenje graničnih vrednosti aeropolutanata i prosečnim mesečnim vrednostima meteoroloških parametara određena je pomoću Pirsonovog testa u programu SPSS. **Rezultati.** Ispitivanu populaciju činile su 1 624 odrasle osobe obolele od astme ili HOBP koje su tokom perioda opservacije (2011. godina) imale ukupno 570 epizoda značajnog (umerenog ili teškog) pogor-

Correspondence to: Ivan Stevanović, Department of Internal Medicine, General Hospital "St.Luke", Prince Mihailo Street 51, 11 300 Smederevo, Serbia. E-mail: ivan12sd@gmail.com

šanja bolesti. Pogoršanje astme bilo je značajno češće kod žena nego kod muškaraca. Broj dana u mesecu sa visokim nivoima suspendovanih čestica $PM_{2.5}$ bio je u statistički značajnoj pozitivnoj korelaciji sa ukupnim brojem umerenih i teških epizoda pogoršanja obe bolesti (astme i HOBP) kod žena. Takođe, značajna povezanost utvrđena je sa brojem umerenih epizoda pogoršanja astme u podgrupama nepušača i gojaznih bolesnika. Pozitivna korelacija mesečnog broja dana sa prekoračenjem granične vrednosti za PM_{10} i pogoršanja bolesti utvrđena je samo za podgrupu gojaznih nepušača sa astmom. Nije nađena značajna povezanost aerozagađenja suspendovanim česticama i egzacerbacija HOBP. Analiza povezanosti sa ambijentalnom temperaturom pokazala je postojanje negativne korelacije sa brojem pogoršanja astme u kategorijama gojaznih žena i nepušača. Povišen atmosferski pritisak bio je u korelaciji sa porastom broja umerenog pogoršanja HOBP kod žena pušača. **Zaključak.** Izloženost suspendovanim česticama, posebno frakciji $PM_{2.5}$, i niskoj temperaturi vazduha može biti pokretač pogoršanja astme koja zahteva urgentno lečenje. Najosetljivije mogu biti žene i gojazni bolesnici.

Ključne reči:
vazduh, zagađenje; čestice, veličina; vreme (klima); bolest, progresija; faktori rizika; pluća, opstruktivne bolesti, hronične; astma.

Introduction

The adverse health effects of air pollution are widely acknowledged. Because the exhaust of steel plant operating in the town of Smederevo may contain a high concentration of pollutants, the quality of air was continuously monitored in recent years. Fossil fuel or coal combusting heating system in the city and aged vehicles also contribute to the pollution. Accordingly, high levels of particulate matter (PM) in the air have been measured there over the past six years.

Epidemiological studies during the last few decades have consistently shown that ambient air particles, even at moderate and low concentrations, can have both short- and long-term effects on health [1]. PM urban air pollution is associated with the increase of emergency visits and hospital admissions due to respiratory diseases like asthma and chronic obstructive pulmonary disease (COPD) [2].

Ambient PM is a mixture of particles generated from different processes, having variable sizes and chemical composition. Current ambient air quality monitoring in many countries, including Serbia, quantifies two different classes of airborne particles according their size – coarse, categorized as PM_{10} (with diameter from 2.5 μm to 10 μm) and fine, labelled as $PM_{2.5}$ (with diameter up to 2.5 μm). Ultrafine particles of nanoscale size (less than 100 nm in diameter) are not routinely monitored. In reality, all fractions are more or less present in the air, and it is still unclear which fraction of PM (coarse, fine or ultrafine) represents the highest risk for public health [3]. In studies of asthma and COPD hospital admissions, coarse PM has a stronger or as strong short-term effect as fine PM [4]. There are also studies indicating the relevance of ultrafine particles in terms of adverse respiratory health effects [5].

The aim of this study was to assess associations between airborne exposures to PM ($PM_{2.5}$ or PM_{10}) and exacerbation of asthma or COPD in adult citizens of Smederevo living in the area surrounding the steel factory.

Methods

This study was conducted in the secondary care General Hospital in Smederevo covering approximately 81,000 inhabitants living in the area of 7 km around the automatic station for air quality monitoring located in Radinac, nearby the steel factory. Data on meteorological conditions (average daily values of temperature, air pressure and wind speed) and air pollutants were collected from the same station, equipped with a Horiba analyser for SO_2 and NO_2, and a Grimm sampler for PM (all records on 2 m height).

The study population consisted of persons registered and followed up as asthma suffering patients (n = 1,100) and COPD patients (n = 524). The patients younger than 18 years were excluded from the study. Data were obtained from medical records. Criteria for moderate or severe exacerbation of the disease were requirements for additional therapy in Outpatients Emergency Room (orally or parenterally administered corticosteroid preparation for 2–12 days), or admittance to hospital, respectively.

For moderate exacerbations, correlations with air pollutants or with meteorological conditions were separately investigated for the subgroups made by the diagnosis (all the patients and separately asthma/COPD patients), by gender, by smoking status, and by body-mass index (BMI) (obese or non-obese). For the patients with severe exacerbations, data on smoking status and BMI were not available. The period of observation was one year (2011).

Independent samples Student's *t*-test was used to test the significance of the difference between the groups. χ^2 test was used to test the significance of the difference between frequencies of exacerbations. All variables were tested with Kolmogorov Smirov test in order to check the normality of their distribution. The correlation between the selected parameters was tested with parametric (Pearson's) bivariate correlation test in program SPSS (Statistical Package for the Social Sciences) software (version 10, SPSS Inc. Chicago, IL) with the complex sample module. A significance level of $p < 0.05$ was considered statistically significant based on two-tailed tests.

Results

The recorded values of air pollutants, meteorological conditions and number of days with air pollutants limit level exceedence are shown in Table 1.

According the recorded values, the average day concentrations of gasses SO_2 and NO_2 in Smederevo have been under 24 h-limit value during the whole year. Therefore, levels of SO_2 and NO_2 were not further considered in terms of association with diseases exacerbations.

Table 1

Meteorological conditions, air pollutants levels and the number of asthma or chronic obstructive pulmonary disease (COPD) exacerbation in Smederevo during 2011

Variable	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Meteorological conditions ($\bar{x} \pm SD$)												
temperature (°C)	0.31 ± 5.15	0.09 ± 3.55	7.33 ± 5.93	13.12 ± 5.53	17.34 ± 3.90	21.66 ± 2.37	23.12 ± 3.97	23.57 ± 2.57	21.56 ± 2.54	11.21 ± 4.40	3.28 ± 4.37	4.28 ± 4.10
Air pressure (mbar)	1009.28 ± 3.56	1008.28 ± 6.64	1010.80 ± 6.86	1004.22 ± 3.65	1006.42± 3.36	1002.48± 3.72	999.07 ± 3.86	1003.06± 2.78	1004.79± 4.46	1009.66± 5.10	1014.80± 4.68	1006.74 ± 8.85
wind speed (m/s)	1.43 ± 0.58	1.71 ± 1.13	1.84 ± 0.74	2.21 ± 1.09	1.53 ± 0.54	1.91 ± 0.74	1.47 ± 0.47	1.48 ± 0.67	1.37 ± 0.45	1.65 ± 0.76	1.20 ± 0.76	1.73 ± 0.72
Air pollutants ($\bar{x} \pm SD$)												
SO_2 (μg/m^3)	43.73 ± 24.25	46.16 ± 15.41	34.54 ± 19.09	17.47 ± 8.16	14.58 ± 8.72	10.23 ± 1.99	14.36 ± 8.28	13.59 ± 4.15	18.32 ± 12.98	19.09 ± 5.38	42.72 ± 18.65	39.51 ± 12.74
NO_2 (μg/m^3)	20.50 ± 8.78	20.82 ± 11.60	16.84 ± 7.87	13.34 ± 6.66	13.00 ± 4.55	10.03 ± 3.37	10.97 ± 3.20	12.70 ± 4.07	15.88 ± 4.86	16.31 ± 8.94	19.15 ± 8.96	16.54 ± 4.99
PM_{10} (μg/m^3)	112.84 ± 53.58	111.23 ± 56.46	98.56 ± 57.99	72.52 ± 38.19	54.37 ± 19.87	46.55 ± 16.64	49.69 ± 20.83	63.21 ± 28.77	67.04 ± 21.95	91.52 ± 58.03	170.13 ± 80.56	84.91 ± 38.51
$PM_{2.5}$ (μg/m^3)	98.82 ± 49.20	95.13 ± 41.37	61.33 ± 29.17	33.67 ± 16.96	28.54 ± 7.46	21.07 ± 7.62	22.10 ± 6.83	25.59 ± 8.57	31.01 ± 10.92	57.72 ± 32.33	130.77 ± 60.57	70.22 ± 33.02
Number of days with exceedance of maximal limit level												
PM_{10}*	31	24	25	18	13	9	16	21	23	20	29	26
$PM_{2.5}$†	31	26	27	10	3	2	1	5	9	20	29	25
Number of exacerbations												
Astma	34	27	30	22	8	24	29	16	27	20	27	36
COPD	20	26	33	21	11	28	27	17	24	20	20	23

***According to Serbian Law of air protection 24h-limit value for PM_{10} is 50 μg/L;**
†Serbia has no 24-h limit value for $PM_{2.5}$. Limit value of 35 μg/ L is set according to the United States Enviromental Protection Agency (EPA) recommendation (available at www.epa.gov/air/criteria.html);
PM – particulate matter; SD – standard deviation; $\bar{x}$ – mean value.

Of 1,624 recorded patients, there were 807 men (461 with asthma and 346 with COPD) and 817 women (639 with asthma and 178 with COPD). Diseases exacerbation happened to 420 patients (209 males and 211 females). The total number of exacerbations was 570, with a significantly higher incidence of worsening of COPD (270/524) than of asthma (300/1100), $\chi^2 = 90.59$, $p < 0.001$. The number of exacerbations *per* patient was 1–6 (moderate), and 1–4 (severe). There were significantly more moderate (442) than severe (128) exacerbations ($p < 0.01$). Table 2 shows the incidence of exacerbations *per* severity and *per* diagnosis. The maximal number of exacerbations (n = 63) was recorded in March, and minimal in May (n = 19).

The mean age of patients with exacerbations was 60.2 ± 15.4 years. There were no statistically significant differences between genders in terms of age (males 61.2 ± 16.8 years, females 59.1 ± 13.9 years). The COPD patients with the mean age of 66.0 ± 12.2 were significantly older than asthma patients with the mean age of 54.8 ± 16.1 years ($t = 9.28$, $p < 0.001$).

In both groups of patients, with asthma and with COPD, there were more moderate than severe episodes of exacerbations. The incidence of moderate exacerbations was significantly greater in the group of patients with asthma than in the group of COPD patients and reversely, severe exacerbations were more frequent in the group of COPD pa-

Table 2

Incidence of asthma and chronic obstructive pulmonary disease (COPD) exacerbations *per* month

Type of exacerbation	Number of exacerbation episodes *per* month				
	n	min	max	$\bar{x} \pm SD$	median
Total number	570	19	63	47.17 ± 12.28	51.5
asthma exacerbations	300	8	36	24.67 ± 7.71	26.0
COPD exacerbations	270	11	33	22.50 ± 5.71	22.0
Moderate exacerbations	442	9	55	36.50 ± 12.41	38.1
asthma moderate exacerbations	267	4	35	21.92 ± 8.21	24.0
COPD moderate exacerbations	175	5	21	14.58 ± 5.30	14.5
Severe exacerbations	128	4	16	10.67 ± 3.58	10.0
asthma severe exacerbations	33	1	5	2.75 ± 1.48	2.5
COPD severe exacerbations	95	3	12	7.92 ± 2.75	7.0

tients ($p < 0.001$). Among the patients with moderate exacerbations, the ratio between the smokers and non-smokers was 138/432, and the ratio between the obese and non-obese patients was 297/273.

Asthma exacerbations were significantly more frequent in women than in men ($f_{asthma\ male} = 97/461$; $f_{asthma\ female} = 203/639$; $\chi^2 = 15.0$, $p < 0.001$). There was no difference in the incidence of COPD exacerbations between genders ($f_{COPD\ male} = 181/346$; $f_{COPD\ female} = 89/178$; $\chi^2 = 0.16$, $p = 0.6$).

The total number of exacerbations *per* month (moderate and severe episodes of both COPD or asthma), was not associated with any of air pollution or weather parameters. However, when considering the subgroups of patients made by gender, a significant association ($p = 0.048$) was proved between the number of days with the exceedance of $PM_{2.5}$ limit level and the total number of exacerbations in the subgroup of female patients. For the subgroups created by the diagnosis (COPD or asthma), a borderline significant association ($p = 0.05$) was shown between the number of days with the excess of $PM_{2.5}$ limit level and exacerbation episodes in the patients with asthma. Further analysis revealed that this association became stronger when considering the following subgroups with moderate episodes of asthma exacerbations: non-smokers ($p = 0.029$), female non-smokers ($p = 0.028$), female patients ($p = 0.020$), obese patients ($p = 0.015$), obese females ($p = 0.011$), and obese non-smokers ($p = 0.010$). The correlations between the number of days with $PM_{2.5}$ exceedance and the number of asthma exacerbations *per* month in various subgroups of patients are shown in Figure 1.

The number of days *per* month with the excess levels of PM_{10} was in a positive correlation with asthma worsening only for a subgroup consisted of obese, non-smoking patients with moderate exacerbations ($p = 0.044$). The association between the number of days of PM_{10} exceedance and the total number of asthma exacerbations was close to, but did not achieve statistical significance ($p = 0.053$). A similar result was obtained when testing asthma exacerbations among females ($p = 0.054$).

Considering meteorological conditions, the average temperature was in a negative borderline significant correlation with the total number of asthma exacerbations in females. A statistically significant negative correlation of temperature and moderate asthma exacerbation was found for the subgroups of obese females ($p = 0.024$), and obese, non-smoking patients ($p = 0.039$).

The number of COPD exacerbations was not associated with air pollution or meteorological conditions. The exception was a positive correlation between the moderate exacerbations of COPD in the female smokers and the increase of the mean barometric pressure.

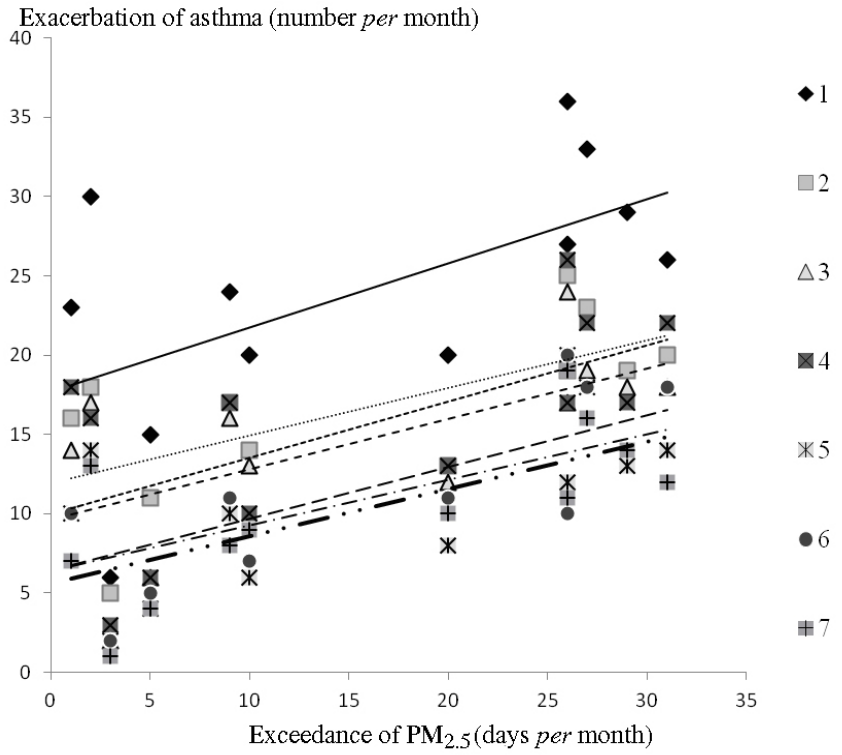


Fig. 1 – Correlations between the number of days with $PM_{2.5}$ exceedance and the number of asthma exacerbations *per* month in various subgroups of patients.

1. Asthma and chronic obstructive pulmonary disease (COPD) females with moderate and severe exacerbations; 2. Asthma females with moderate and severe exacerbations; 3. Asthma females with moderate exacerbations; 4. Asthma non-smokers with moderate exacerbations; 5. Asthma non-smoking females with moderate exacerbations; 6. Asthma obese non-smokers with moderate exacerbations; 7. Asthma obese females with moderate exacerbations.

PM – particulate matter.

The subgroups of patients in a statistically significant or borderline correlation with air pollution or meteorological parameters are listed in Table 3.

Because of limited observational period of just 12 months, reliable statistical analysis of season incidence was not possible.

For the two subgroups of patients (obese females and obese non-smokers with moderate asthma exacerbations) for which the significant association between both ambient temperature and air pollution was revealed, we compared the incidence of disease exacerbation between the coldest and the warmest 4 months of the year, as well as between the 4 months with the highest and the lowest number of days with the excessive levels of $PM_{2.5}$. The coldest months were January, February, November and December, and the warmest was a 4-month period from June to September (Table 1). The incidence of moderate asthma exacerbation was lower during the summer than the winter months, but there was no statistically significant difference. In obese females and obese non-smokers, average number of exacerbation *per* month was 8.0 ± 3.7 in the warmest months *vs* 14.0 ± 3.5 in the coldest months ($t = 2.32$, $p = 0.059$) and 9.7 ± 3.4 in the warmest months *vs* 15.5 ± 4.4 in the coldest months ($t = 2.05$, $p = 0.085$), respectively. The months with the highest number of days with over the limit concentration of $PM_{2.5}$ were January, February, March and November. Air pollution was lowest in the 4-month period from May to August (Table 1). The incidence of moderate asthma exacerbation was significantly lower during the period with the small number of days with the excess of $PM_{2.5}$ than in months with high number of days with a excessive air pollution, for both obese females (6.2 ± 5.1 *per* month *vs* 15.2 ± 2.3; $t = 3.03$, $p = 0.002$) and obese non-smokers (7.5 ± 4.9 *vs* 17.5 ± 2.5; $t = 3.61$, $p = 0.001$).

Discussion

Exacerbations of asthma and COPD are important clinical events, resulting in the decline of lung function, requirement of additional therapy, hospitalisation, or even death. For patients with asthma, exacerbations and poor control can be the result of exposure to environmental triggers, such as allergens and air particulates [6]. It is well-known that cigarette smoking is responsible for the vast majority of COPD. However, other sources of exposure, including working or living in polluted indoor and outdoor environments are recognized as significant contributors to the development, progression, and exacerbation of COPD [7].

In our study, asthma exacerbations were significantly more frequent in females than in males. These results are consistent with findings from many epidemiological studies suggesting that adult women suffer from more severe asthma than men. Gender differences appear to be the product of biological properties including body characteristics, hormonal, sociocultural and environmental differences [8]. Our analysis revealed that the number of days with excess levels of $PM_{2.5}$ *per* month was in positive correlation with the number of both moderate and severe exacerbation episodes among asthma patients (borderline significance, $p = 0.05$), and especially among females ($p = 0.02$). Other studies have also shown that PM of 10 μm or less seemed to affect primarily women with asthma, even at levels even below the national air quality standards [9].

Our further analysis has shown that there was the highest positive correlation between the number of days *per* month with exceedance of $PM_{2.5}$ and the number of moderate asthma exacerbations in obese patients. Other studies [10] suggested that BMI is a strong predictor of incident asthma among adult women. Furthermore, a meta-analysis of prospecti-

Table 3

Single correlation coefficients between the number of disease exacerbations *per* month and air characteristics in the selected subgroups of patients

Variables	Patients with exacerbations	*r*	*p*
Number of days with excess of $PM_{2.5}$	Female asthma and COPD patients, moderate and severe exacerbations	0.581	0.048
	Asthma patients, moderate and severe exacerbations	0.576	0.050
	Asthma females, moderate and severe exacerbations	0.657	0.020
	Asthma females, moderate exacerbations	0.631	0.028
	Asthma non-smokers, moderate exacerbations	0.628	0.029
	Asthma female non-smokers, moderate exacerbations	0.630	0.028
	Asthma obese patients, moderate exacerbations	0.677	0.015
	Asthma obese non-smokers, moderate exacerbations	0.708	0.010
	Asthma obese females, moderate exacerbations	0.700	0.011
Number of days with excess of PM_{10}	Asthma females, moderate and severe exacerbations	0.568	0.054
	Asthma obese non-smokers, moderate exacerbations	0.589	0.044
	Asthma patients, moderate and severe exacerbations	0.570	0.053
Mean temperature	Asthma females, moderate and severe exacerbations	-0.577	0.050
	Asthma females, moderate exacerbations	-0.576	0.050
	Asthma obese non-smokers, moderate exacerbations	-0.601	0.039
	Asthma obese females, moderate exacerbations	-0.645	0.024
Mean air preessure	COPD female smokers, moderate exacerbations	0.580	0.048

COPD – chronic abstructive pulmonary disease; PM – particulate matter.

ve epidemiologic studies revealed that overweight and obesity are associated with incident asthma in both women and men [11]. Association of obesity with asthma exacerbations is not well understood, as some co-factors like social status, smoking or gastroesophageal reflux disease (GERD) may be involved. After adjusting for demographics, smoking status, oral corticosteroid use, evidence of GERD, and inhaled corticosteroid use, Mosen et al. [12] suggested that obesity is associated with worse asthma outcomes, especially with an increased risk of asthma-related hospitalizations.

The mechanisms underlying the relationship between obesity and asthma have not been fully established, but adipose tissue-derived hormones have certain role in pathogenesis and control of asthma [13, 14]. A link between obesity, asthma and air pollution may be the finding that adults who are obese or even overweight, actually breathe in 7–50% percent more air *per* day than adults at healthier weights which makes them more vulnerable to air contaminants causing asthma and other pulmonary diseases [15].

Our analysis of the association between excess PM_{10} and asthma exacerbation revealed a positive correlation only in case of asthma obese non-smokers with moderate exacerbations.

The obese patients were also more vulnerable to the meteorological conditions, as ambient temperature drop was associated with the increase in the number of asthma attacks. The incidence of moderate exacerbations was higher during the coldest months than during the summer in the obese females and non-smokers, but there was no statistically significant difference. A low inverse correlation of ambient temperature with the attendance for asthma attacks during a year on similar number of patients (232) was reported by Rossi et al [16]. Their conclusions are that air pollen levels are not associated with asthma exacerbation, temperature among the meteorological factors had a small association, but increased levels of pollutants, especially NO_2, are associated with attacks of asthma. In our study of air pollution effects, air pollen levels were not available and NO_2 was not considered because it was within acceptable limits, but there was a significantly higher number of asthma exacerbations in months with excessive level of $PM_{2.5}$ than in the period when there has been little air pollution. Other studies on a much larger number of patients show that cold temperature is related to the increased risk of significant exacerbation of asthma in adults [17] and children [18].

In other studies indicate that acute exacerbations of COPD is associated with a short-term exposure to air pollution [19], and PM_{10} show a strong association with adverse respiratory health effects, even when adjusted for other major risk factors such as cigarette smoking [20]. However, in our study, though the total number of COPD exacerbations was not significantly lower than asthma exacerbations (270 and 300, respectively), a connection between high concentrations of PM ($PM_{2.5}$ or PM_{10}) and COPD exacerbation was not proven.

The number of COPD exacerbations was in a positive correlation only with the increased barometric pressure. Unfortunately, we had no validated data on air humidity. The association between barometric pressure and the increased number of COPD exacerbations has been shown by authors from different climate areas. Thus, a study from Taiwan [21] suggests higher barometric pressure, more hours of sunshine and lower humidity to be positively correlated with an increase in COPD consultations, while German authors [22] find barometric pressure to be in positive, but solar radiation in negative correlations with COPD exacerbations. Cold temperature is an important environmental factor for respiratory diseases exacerbations and COPD attacks are more frequent in winter [23]. However, it was not shown in our study.

The limitations of this study include the lack of some relevant information in the hospital database on severe exacerbations, such as smoking status and BMI, as well as short period of observation resulting in relatively small number of patients in certain subgroups. Nevertheless, this study demonstrated association of PM air pollution, especially $PM_{2.5}$, and asthma exacerbation. Females and obese patients were the most vulnerable subpopulations.

Conclusion

The results obtained in this study indicate that in the town of Smederevo, exposure to airborne particles, mainly to those with diameter up to 2.5 μm, and low temperature may trigger asthma exacerbations requiring emergency care. Reducing air pollution may lead to health benefits, especially among certain groups of asthmatic patients.

REFERENCES

1. *Katsouyanni K.* Ambient air pollution and health. Br Med Bull 2003; 68(1): 143−56.
2. *Halonen J, Lanki T, Yli-Tuomi T, Kulmala M, Tiittanen P, Pekkanen J.* Urban air pollution, and asthma and COPD hospital emergency room visits. Thorax 2008; 63(7): 635−41.
3. *Karakatsani A, Analitis A, Perifanou D, Ayres JG, Harrison RM, Kotronarou A,* et al. Particulate matter air pollution and respiratory symptoms in individuals having either asthma or chronic obstructive pulmonary disease: a European multicentre panel study. Environ Health 2012;11(1): 75.
4. *Brunekreef B, Forsberg B.* Epidemiological evidence of effects of coarse airborne particles on health. Eur Respir J 2005; 26(2): 309−18.
5. *Peters A, Wichmann H, Tuch T, Heinrich J, Heyder J.* Respiratory effects are associated with the number of ultrafine particles. Am J Respir Crit Care Med 1997; 155(4): 1376−83.
6. *Vernon MK, Wiklund I, Bell JA, Dale P, Chapman KR.* What do we know about asthma triggers? a review of the literature. J Asthma 2012; 49(10): 991−8.
7. *Ko FW, Hui DS.* Air pollution and chronic obstructive pulmonary disease. Respirology 2012; 17(3): 395−401.
8. *Melgert BN, Ray A, Hylkema MN, Timens W, Postma DS.* Are there reasons why adult asthma is more common in females. Curr Allergy Asthma Rep 2007; 7(2): 143−50.
9. *Meng Y, Wilhelm M, Rull RP, English P, Ritz B.* Traffic and outdoor air pollution levels near residences and poorly controlled

asthma in adults. Ann Allergy Asthma Immunol 2007; 98(5): 455−63.

10. *Beuther DA, Sutherland E.* Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. Am J Respir Crit Care Med 2007; 175(7): 661−6.
11. *Assad N, Qualls C, Smith LJ, Arynchyn A, Thyagarajan B, Schuyler M,* et al. Body mass index is a stronger predictor than the metabolic syndrome for future asthma in women The longitudinal CARDIA study. Am J Respir Crit Care Med 2013; 188(3): 319−26.
12. *Mosen DM, Schatz M, Magid DJ, Camargo CA.* The relationship between obesity and asthma severity and control in adults. J Allergy Clin Immunol 2008; 122(3): 507−11.
13. *Schaub B, von Mutius E.* Obesity and asthma, what are the links. Curr Opin Allergy Clin Immunol 2005; 5(2): 185−93.
14. *Tsaroucha A, Daniil Z, Malli F, Georgoulias P, Minas M, Kostikas K,* et al. Leptin, adiponectin, and ghrelin levels in female patients with asthma during stable and exacerbation periods. J Asthma 2013; 50(2): 188−97.
15. *Brochu P, Bouchard M, Haddad S.* Physiological daily inhalation rates for health risk assessment in overweight/obese children, adults, and elderly. Risk Anal 2014; 34(3): 567−82.
16. *Rossi O, Kinnula V, Tienari J, Huhti E.* Association of severe asthma attacks with weather, pollen, and air pollutants. Thorax 1993; 48(3): 244−8.
17. *Abe T, Tokuda Y, Ohde S, Ishimatsu S, Nakamura T, Birrer RB.* The relationship of short-term air pollution and weather to ED visits for asthma in Japan. Am J Emerg Med 2009; 27(2): 153−9.
18. *Guo Y, Jiang F, Peng L, Zhang J, Geng F, Xu J,* et al. The Association between Cold Spells and Pediatric Outpatient Visits for Asthma in Shanghai, China. PLoS ONE 2012; 7(7): e42232.
19. *Atkinson R, Anderson H, Sunyer J, Ayres J, Baccini M, Vonk J,* et al. Acute effects of particulate air pollution on respiratory admissions: results from APHEA 2 project Air Pollution and Health: a European Approach. Am J Respir Crit Care Med 2001; 164(10): 1860−6.
20. *Schwartz J.* Short term fluctuations in air pollution and hospital admissions of the elderly for respiratory disease. Thorax 1995; 50(5): 531−8.
21. *Tseng C, Chen Y, Ou S, Hsiao Y, Li S, Wang S,* et al. The effect of cold temperature on increased exacerbation of chronic obstructive pulmonary disease: a nationwide study. PLoS ONE 2013; 8(3): e57066.
22. *Ferrari U, Exner T, Wanka ER, Bergemann C, Meyer-Arnek J, Hildenbrand B,* et al. Influence of air pressure, humidity, solar radiation, temperature, and wind speed on ambulatory visits due to chronic obstructive pulmonary disease in Bavaria, Germany. Int J Biometeorol 2012; 56(1): 137−43.
23. *Jenkins C, Celli B, Anderson J, Ferguson G, Jones P, Vestbo J,* et al. Seasonality and determinants of moderate and severe COPD exacerbations in the TORCH study. Eur Respir J 2012; 39(1): 38−45.

Received on November 11, 2014.
Revised on January 15, 2015.
Accepted on January 26, 2015.
Online First April, 2015.

ORIGINAL ARTICLE

UDC: 616.12-056.7:616-036.22
DOI: 10.2298/VSP140917033M

The incidence of congenital heart defects in the world regarding the severity of the defect

Incidencija urođenih srčanih mana u svetu prema težini mane

Vesna Miranović

Institute for Children's Disease, Clinical Center of Montenegro, Podgorica, Montenegro

Abstract

Bacground/Aim. Congenital heart defects (CHDs) are structural or functional abnormalities of the heart present at birth even if they are detected much later. Their importance lies in the fact that, depending on the severity, they change the quality of life, and may be life threating. In addition, we should not ignore the high costs of treating people with congenital heart disease. The aim of this study was to analyze the incidence of congenital heart disease in relation to the severity in the world based on the available literature. **Methods.** All the available literature on the incidence of CHD cases regarding the severity of CHD published from 1955 to 2012 was analyzed. The researcher was able to read the titles and abstracts of 128 papers on the subject. Due to methodological inconsistency, 117 of the papers were rejected. Based on the criteria of reliability, availability and comparability, our analysis included 11 studies testing CHD incidence regarding the severity of the defect conducted all over the world. The Yates' χ^2-test was used to compare the observed incidences. **Results.** The frequency of severe congenital heart defects, ranged from 0.414 to 2.3/1,000 live births, the incidence of moderate congenital heart defects from 0.43 to 2.6/1,000 live births while in the group of minor congenital heart defects the incidence ranged from 0.99 to 10.3/1000 live births. There were no statistically significant differences in the incidence of mild, moderate and severe CHDs. **Conclusion.** The results obtained studying of the available data suggest that no statistically significant difference in the incidence of mild, moderate and severe congenital heart defects. A universal methodological approach to the incidence of CHD is essential.

Key words:
heart defects, congenital; incidence; severity of illnes index; epidemiology; statistics.

Apstrakt

Uvod/Cilj. Urođene srčane mane su strukturne ili funkcionalne anomalije srca prisutne na rođenju čak iako se otkriju mnogo kasnije. Njihov značaj leži u činjenici da zavisno od težine menjaju kvalitet života, ili ga ozbiljno ugrožavaju. Osim toga, ne treba zanemariti ni visoke troškove lečenja osoba sa urođenim srčanim manama. Cilj rada bio je da se analiziraju vrednosti incidencije urođenih srčanih mana u odnosu na njihovu težinu u svetu na osnovu dostupne literature. **Metode.** Analizirana je sva dostupna literatura koja se odnosi na incidencije urođenih srčanih mana prema težini objavljena u periodu od 1955. do 2012. godine. Analizirano je 128 naslova i apstrakata na ovu temu. Zbog metodološke nedoslednosti, 117 radova je odbačeno. Na osnovu kriterijuma dostupnosti i uporedivosti, u našu analizu uključeno je 11 studija u cilju proučavanja incidencije urođenih srčanih mana prema težini. Pri poređenju proučavanih incidencija korišten je Yates-ov χ^2-test. **Rezultati.** Učestalost teških urođenih srčanih mana kreće se u rasponu od 0,414 do 2,3/1 000 živorođene dece, incidencija srednje teških urođenih srčanih mana je u intervalu od 0,43 do 2,6, dok je u grupi lakih mana incidencija u rasponu od 0,99 do 10,3/1 000 živorođene dece. Nije rađena statistički značajna razlika u incidenciji lakih, srednje teških i teških urođenih srčanih mana. **Zaključak.** Rezultati dobijeni na osnovu dostupnih podataka navode na zaključak da nema statistički značajne razlike u incidencijama lakih, srednje teških i teških urođenih srčanih mana kao i da je neophodan univerzalni metodološki pristup izučavanju incidencije urođenih srčanih mana.

Ključne reči:
srce, kongenitalne mane; incidenca; bolest, indeks težine; epidemiologija; statistika.

Introduction

Congenital heart defects (CHDs) are structural or functional abnormalities of the heart present at birth even if they are detected much later. CHDs are the most common birth defects that occur with an incidence of 4–10/1000 live births [1]. If one takes into account the fact that was recently published by the World Health Organization, that the incidence of CHDs is 10 per 1,000 live births, it is clear that each year 1.5 million children with CHDs are born in the world [2]. The fact that CHDs cause death by 10% of children in the first year of life shows their impact on overall

Correspondence to: Vesna Miranović, Institute for Children's Disease, Clinical Center of Montenegro, Ljubljanska bb, Podgorica, Montenegro. Phone: 00382 20 412 716. E-mail: vesmir@t-com.me

mortality [3]. Not long ago all children with aortic atresia died in the first few months of life, 85% of children with transposition of the great arteries in the first few days after birth, and 78% with pulmonary atresia died during the first 6 months of life [4]. Children with CHDs occupy 25–30% of intensive care facilities.

The past 5 decades have witnessed extraordinary advances in diagnostics and treatment of patients with CHDs from a puzzling curiosity to more or less solved problem. Today almost 85% of babies born with CHD can expect to reach adulthood [5].

The importance of CHDs lies in the fact that, depending on the severity, they change the quality of life, or may be seriously life threatening requiring urgent measures in terms of organization of early diagnosis, well healthcare access and prompt medical care action.

Awareness of researchers on the incidence of CHDs is gradually maturing. First, the subject of the study was the incidence of CHDs itself, then the incidence of each CHD, then the focus of research was directed toward defects with the most severe clinical symptoms and the need for cardiac surgery treatment. CHD classification in the categories of severity (severe, moderate and mild CHDs) is not only a didactical issue, but a multidimensional approach including: use of health service, health related quality of life, as well as psychological state and social relationship. Mild (simple, trivial) CHDs do not significantly affect the life pattern of a patient or the quality of his/her life. Apparently innocent, harmless, minor CHDs need to be monitored as well. Studying of their incidence can point to some of the causes of CHDs or convince us of the sensitivity and specificity of new diagnostic tools.

However, there is a special interest in the study of the incidence of moderate (significant) and severe (complex) CHDs because they represent a very important health problem. The fact that the number of children and adults with CHDs is constantly increasing, generally raises medical attention. Huge advances in pediatric intensive care, echocardiography and cardiac surgery have provided an enormous growth in the number of surviving children and adults with CHDs, so that today there are more adults than children with CHDs. This continually growing population presents a big challenge for cardiologists and even more for the health system because of its unique issues and needs. Since a longer life does not necessarily mean a better life, there is a growing consensus that it is necessary to offer them specialized care facilities to meet their needs. The results of studies on the incidence of CHDs regarding to their severity should be used to provide data to help in proper direction of resource allocation in CHDs.

Methods

All the available literature on the incidence of CHD regarding the severity of CHD published from 1955 to 2012 was analyzed. Following the example of Hoffman and Kaplan [6], we took the year 1955 as the lower limit when cardiac catheterization was becoming a common diagnostic test. Thus, the researcher was able to read the titles and abstracts of 128 papers on the subject. Due to methodological inconsistency 117 of the papers were rejected. Based on the criteria of reliability, availability and comparability, our analysis included 11 studies testing the incidence of CHD conducted all over the world.

The following inclusion criteria were necessary to be fulfilled to be considered in this analysis: to have adopted the definition for CHDs by Mitchell [7], to have adopted and applied the division of CHDs in relation to the severity to mild (simple, trivial), moderate (significant) and severe (complex); applied acceptable diagnostic hierarchy ("physiological" or "two-dimensional"); a clearly defined territory and population; that CHDs were diagnosed in the first 12 months of life; children with suspected CHD examined by pediatric cardiologist; the study lasted for at least 5 years (the only exception was a study in Beijing [8]).

The Yates' χ^2-test was used to compare the observed incidences. The incidence was compared within each group separately (minor, moderate, severe), starting from the premise that there was no statistically significant difference among the analyzed samples. The arithmetic mean value of the incidence that is hypothetical and known in advance, was the value to the incidence that came in the "New England Regional Infant Cardiac Program," which is 1.5/1,000 live births for severe CHDs, 2.5/1,000 live births for moderate and 2.2/1,000 live births for minor CHDs [9].

Results

Eleven studies included over more than 8 million live births and about 40,000 diagnosed CHDs were analyzed. The important characteristics of the studies are given in Table 1.

Table 1

Important characteristics of the studies included in the analysis

Country	Duration (years)	Live births (n)	Cases with CHDs	Incidence of CHD *per* 1,000 live births
Massachusetts, USA	6	1,528,964	8,071	6.2
Clevelend, USA	13	477,960	2,671	5.59
Montenegro	10	88,098	1,004	8.8
Taiwan	6	238,143	3,115	13.08
Tuzla, Bosnia	6	39,699	234	6.12
BejJing, China	1	84,062	686	8.2
Anatolia, Turkey	8	219,870	1,693	7.77
Quebec, Canada	15			5.78
Island	10	44,013	740	9.2
Atlanta, USA	20	937,195	5,813	5.8
France	10	4,400,000	12,932	2.85

The incidence of CHDs in dependence on their severity is given in Table 2.

The results showed that the incidence of severe CHDs was in the range of 0.414 to 2.3/1,000 live births. The highest frequency was in the range of 1.4–1.6/1,000 live births. The incidence of moderate CHD is in the range of 0.43–2.6, but even 9 studies reported the frequency interval 1.97–2.6, while in the minor group CHD incidence ranged from 0.99–10.3/1,000 live births. The percentage of different categories of CHD severity relative to the total incidence for severe CHDs was found to be from 5.38% to 50.2%, while the minor and moderate ranged from 15.6% to 38.6% and from 34.5% to 74.64% (Table 2).

Table 2

Incidence of congenital heart defects (CHDs) *per* 1,000 live births regarding the severity in 11 different studies

Place of study	Minor CHDs		Moderate CHDs		Severe CHDs	
Massachusetts, USA,	2.2		2.5		1.5	
Clevelend, USA,	3.136		2.04		0.414	
Montenegro	5.59		2.6		0.61	
Taiwan	10.3	$\chi^2 = 11.698$*	1.99	$\chi^2 = 1.367$*	1.5	$\chi^2 = 0.718$*
Tuzla, Bosnia	2.4	$p = 0.2309$	2.26	$p = 0.9980$	1.46	$p = 0.9998$
BejJing, China	4.96		2.34		0.9	
Anatolia, Turkey	4.63		1.97		1.1	
Quebec, Canada	2.23		2.1		1.45	
Island	9.2		4.6		2.3	
Atlanta, USA	3.05		2.11		0.64	
France	0.99		0.43		1.43	

***Statistically insignificante difference ($p > 0.05$, Yates' χ^2-test); Incidence – number of new cases of CHD *per* 1,000 live births.**

Based on the hypothesis that most of research authors on the incidence expressed the view that the incidence of CHDs is predictable in relation to the severity of defects, in fixed ranges, we found it reasonable for our hypothesis to be based on this presumption. Using the Yates' χ^2-test we came to the result that supports the hypothesis of no statistically significant differences among the incidence of minor ($\chi^2 = 11.698$; $p = 0.2309$), moderate ($\chi^2 = 1.367$; $p = 0.9980$) and severe ($\chi^2 = 0.718$; $p = 0.9998$) CHDs (Table 2).

Discussion

Studying of the incidence of CHD requires a series of complex procedures that are tailored to the specific phenomena investigated: some CHDs are very rare, others have special natural evolution, and the third group shows sometimes variations of clinical manifestations. On the other hand, the reliability of each epidemiological study depends on a clear set of definitions, including population, methods used and applied, ethical principles, inclusion of rigorous criteria is mandatory for each study, time of diagnosis, classification, diagnoses faults, their clustering in relation to severity. Comparison of the data is only possible if there is a methodological compliance of each listed segment.

This analysis is the result of extensive research that has led us to the conclusion that we must not succumb to the imperative of including in the study just any article that concerns the problems of the incidence of CHD in relation to the severity of the defect. Despite the large number of studies that have reviewed, only a small number of them (n = 11) met the criteria for inclusion in the study. Nevertheless, we believe that the applied methodological procedure for conducting the research upon which we drew the conclusions is more important than the number of studies included to the analysis. Of course, all provided that the survey covers the population large enough to make conclusions that are considered reliable. A meta-analysis published in 2011 assures us that our presumptions are accurate. The authors that drew their study's conclusions based on 114 papers published on the topic of the prevalence of CHDs, when commenting critically on their study limitations expressed a degree of doubt in their performance. They state that it is very hard to determine whether the detected differences in CHD birth prevalence are real or merely methodological [10].

We felt it reasonable to include in the analysis those studies that covered the period of 5 years or longer. The study should last for many years to measure the level of occasional high or low values of incidence. The only exception was made in a research conducted in Beijing, given the large number of live births in that city on annual basis and a very small amount of data on this subject from the Far East [8].

In this analysis older studies [9, 11], and recent studies [8, 12–18] are equally represented because this was an opportunity for confronting old and new diagnostics and testing their impact on the incidence of CHD regarding the severity of the defect. The result showed that no substantial, statistically significant difference was found in the groups of CHD, and the right opportunity to realistically define the impact of echocardiography on epidemiological aspect of severe, moderate and mild CHD. There is a general agreement that echocardiography fundamentally affected the content of the research of CHD incidence results. Modern studies (from around 1985 to nowadays), using echocardiography have improved the knowledge of the frequency of CHDs. But Hoffman and Kaplan [6], with the authority of great researchers in this area, conclude that the incidence of severe and moderate CHD has not changed substantially in the last

50 years anywhere in the world. They believe that echocardiography does not contribute significantly to the diagnosis of severe and moderate CHDs, but the influence on the growth of minor CHDs, especially in the identification of small muscular ventricular septal defects (VSDs) is important. They said that the frequency of clinical examination from previous studies was high and when combined with catheterization, many children with severe and moderate CHDs were identified with great certainty.

Confirmation of these claims is the fact that the estimated number of adults with severe, moderate or mild CHD in USA was based on the results from the New England Regional Infant Cardiac Program, which is mainly carried out in the time before the invention of echocardiography [9]. Use of echocardiography has influenced the identification of the non-categorized defects that are commonly found at the end of the list of diagnosed CHD called "all others", "mixed group" by reducing their share from 28% [19] to 3.2% [13]. Its noninvasive nature and availability, sensitivity and specificity are the recommendations for widespread use.

The results of our study showed that the prevalence of severe CHD is in the range from 0.414 to 2.3/1,000 live births with the highest frequency in the range of 1.4–1.6/1,000 live births. The incidence of moderate CHD is much more balanced. Even 9 studies reported the frequency interval of 1.97–2.6. Highest diversity is expected to be in the mild CHD group (from 0.99–10.3/1,000 live births), and yet not distinct enough to be statistically significant.

Analyzing the results of statistical processing and the fact that we found no statistically significant difference in the incidence in none of the three groups of CHD, we have focused on the examination of the reasons for this to happen in several directions. First, previous studies that are part of our analysis are not burdened by a large number of harmless, clinically asymptomatic VSDs. Part of recent researches in the incidence analysis included only those VSDs which required more than three clinical examinations of the cardiologist during the first year of life. This way, the incidence of VSD reduced to a reasonable level, and indirectly influenced the minor incidence of CHD.

Share of different severity categories of CHD in relation to the overall incidence has shown a high degree of diversity primarily in the category of severe CHD (from 5.38% to 50.2%). Such a high percentage of severe CHD is attributed to studies that have reported an overall low incidence of CHD, mainly on account of minor CHD, so the percentage share of severe CHD reached high values.

We were faced with several problems while comparing the available studies. The first one was an attitude to the classification of CHD. We had a dilemma about the classification used in analyzed studies to define the criteria for inclusion in the analysis. "New England Regional Infant Cardiac Program" offered a classification based on anatomical significance of CHD [9]. "Baltimore-Washington study" in the classification, gives priority to the components of the earliest embryonic malformation disorder [20], but in practice, however, the most commonly used model takes into account the physiological hierarchy (considers the most significant impediment, the earliest lesion that requires intervention, or that causes the greatest hemodynamic disturbances). For classifying children with CHD in Montenegro, we used a two-dimensional approach for the model applied in the metropolitan Atlanta [21] and Wren et al. [13] from New Castle, United Kingdom, which published the results of their work and offered this kind of diagnostic hierarchy as a standard in 2000. It consolidates and defines the major structural anomalies, and abnormalities that contribute to the clinical recognition of cardiovascular malformations. We considered that the inclusion of those studies that have incorporated the "physiological" or "two-dimensional" approaches in the analysis lead to the most accurate data.

The second problem was source of data: secondary or tertiary health center. In small countries such as Montenegro, Island, Bosnia-Herzegovina it means that diagnosis, registration, and follow-up are conducted by only a few cardiologists, and take place at a single center for pediatric cardiology. In big countries the access to a pediatric cardiologist is possible even on secondary level, but cardiac surgery, cardiac intensive care units are part of tertiary health care. So, there is a high possibility of dissipation of patient data in the way from secondary to tertiary level. For example, studies conducted in Korea in the institution of secondary level, we can see that results are unrepresentative and meaningless [22]. For these reasons, we have included projects that contain detailed description of how the data was collected from the base to the top of the health care system, which has convinced us in the greater coverage of patients with CHD.

The third problem was counting or estimation of patients with CHDs. One major dilemma was whether to include in the analysis those studies whose data is based on statistical methods for estimating the number of patients with different types of CHD. Large health care systems like USA [23], Germany [24], and Great Britain [25] chose the method of extrapolating to estimate the prevalence of severe, moderate and mild CHD based on the results of "New England Regional Infant Cardiac Program." They used a documented incidence of 1.5/1,000 live births for severe, 2.5/1,000 live births for significant, and 2.2/1,000 live births for simple CHD. Considering that the base of each estimate was a certain serious study, methodological impeccably formulated, we have included the results of these studies in the analysis.

It is obvious that awareness of researchers in the incidence of CHD has been maturing gradually: the first subject of study was the incidence of CHD itself, then each individual CHD, and finally the focus of the research was directed towards the defects with the most severe clinical symptoms and cardiac surgery treatment (moderate and severe). After the invention of echocardiography attention was concentrated on a small VSD that previously could not be diagnosed.

The first organized, conceptually arranged approach to examine the incidence of CHDs was "New England Regional Infant Cardiac Program," which includes a number of

hospitals in New England that began to work on improving the quality health services to children with CHD in 1968 and still continues [9]. In this study, we can recognize only remarks of necessity to allocate significant and severe CHD in the group of interest. Of course, the criteria for inclusion in this category correspond to time and former level of knowledge: children with CHD who required cardiac catheterization or cardiac surgery or died with CHD in the first year of life. Several other studies deserve attention because of the introduction of useful innovations in the study of the incidence of CHDs. These studies were conducted in Blackpool in the period from 1957 to 1971 [11] and in Liverpool [26]. A set of CHD diagnoses on the basis of clinical examination time ranges from 52% [13], 44% [26], 32% [9] to 26% [14]. That is the reason that the data collected for more than 30–40 years is less accurate and complete than nowadays, but the approach to the patient was more clinical, less technological.

Hoffman [27] is the most consistent cardiologist in the study of the epidemiological aspects of CHDs. After a great experience in this field he has suggested the introduction of a standard set for the diagnosis of CHDs and standard methods of implementation of studies aimed at determining the incidence of CHDs which would allow data comparability. All important studies after the introduction of echocardiography agree on one thing: there is no change in the incidence of severe and moderate CHDs, but there is a significant increase in mild defects [13, 28]. A study conducted in Montenegro on a sample of 88,098 live births revealed that the incidence of moderate to severe defects in the analyzed period of ten years is without oscillations, while easier defects show temporal variation (increase sharply, then maintain the same high-level and then a lower drop) [29].

Conclusion

The results obtained on the basis of the available data support the hypothesis that there is no statistically significant difference in the incidence of mild, moderate and severe congenital heart defects.

A universal methodological approach to the study of incidence of congenital heart defects is essential.

REFERENCES

1. *Loffredo CA.* Epidemiology of cardiovascular malformations: prevalence and risk factors. Am J Med Genet 2000; 97(4): 319−25.
2. *World Health Organization* . World Health Statistics Annual. Available from: www.thebody.com/content/art61960.html [cited 2011 May 16]
3. *Abu-Harb M, Hey E, Wren C.* Death in infancy from unrecognized congenital heart disease. Arch Dis Child 1994; 71(1): 3–7.
4. *Keith JD* . Prevalence, incidence and epidemiology. In: *Keith JD, Rowe RD, Vlad P* , editors. Heart Disease in Infancy and Childhood. 3rd ed. New York : Macmillan; 1978. p. 3–13.
5. *Kidd BS*. The fate of children with transposition of the great arteries following balloon atrial septostomy.In: *Kidd BS,* editor. The child with congenital heart disease after surgery. Mount Kisco, NY: Futura publishing Co; 1976.
6. *Hoffman JE, Kaplan K.* The incidence of congenital heart disease. J Am Coll Cardiol 2002; 39(12): 1890−900.
7. *Mitchell SC.* The ductus arteriosus in the neonatal period. J Pediatr 1957; 51(1): 12–7.
8. *Yang XY, Li XF, Lü XD, Liu YL.* Incidence of congenital heart disease in Beijing, China. Chin Med J (Engl) 2009; 122(10): 1128−32.
9. *Fyler DC, Buckley LP, Hillenbrand WE.* Report of the New England Regional Infant Cardiac Program. Pediatrics 1980; 65(2 Pt 2): 375−461.
10. *van der Linde D, Konings EE, Slager MA, Witsenburg M, Helbing WA,* et al. Birth prevalence of congenital heart disease worldwide: a systematic review and meta-analysis. J Am Coll Cardiol. 2011; 58(21): 2241−7.
11. *Bound JP, Logan WF.* Incidence of congenital heart disease in Blackpool 1957-1971. Br Heart J 1977; 39(4): 445−50.
12. *Botto LD, Correa A, Erickson JD.* Racial and temporal variations in the prevalence of heart defects. Pediatrics 2001; 107(3): E32.
13. *Wren C, Richmond S, Donaldson L.* Temporal variability in birth prevalence of cardiovascular malformations. Heart 2000; 83(4): 414−9.
14. *Wu MH, Chen HC, Lu CW, Wang JK, Huang SC, Huang SK.* Prevalence of congenital heart disease at live birth in Taiwan. J Pediatr 2010; 156(5): 782−5.
15. *Begić H, Tahirović H, Mesihović-Dinarević S, Ferković V, Atić N, Latifagić A.* Epidemiological and clinical aspects of congenital heart disease in children in Tuzla Canton, Bosnia-Herzegovina. Eur J Pediatr 2003; 162(3): 191−3.
16. *Stephensen SS, Sigfusson G, Eiriksson H, Sverrisson JT, Torfason B, Haraldsson A,* et al. Congenital cardiac malformations in Iceland from 1990 through 1999. Cardiol Young 2004; 14(4): 396−401.
17. *Başpinar O, Karaaslan S, Oran B, Baysal T, Elmaci AM, Yorulmaz A.* Prevalence and distribution of children with congenital heart diseases in the central Anatolian region, Turkey. Turk J Pediatr 2006; 48(3): 237−43.
18. *Marelli AJ, Mackie AS, Ionescu-Ittu R, Rahme E, Pilote L.* Congenital heart disease in the general population: changing prevalence and age distribution. Circulation 2007; 115(2): 163−72.
19. *Feldt RH, Avasthey P, Yoshimasu F, Kurland LT, Titus JL.* Incidence of congenital heart disease in children born to residents of Olmsted County, Minnesota, 1950-1969. Mayo Clin Proc 1971; 46(12): 794−9.
20. *Ferencz C, Loffredo CA, Correa-Villasenor A, Wilson PD.* Perspectives in Pediatric Cardiology, Volume 4: Epidemiology of Congenital Heart Disease: The Baltimore-Washington Infant Study: 1981-1989. Armonk, NY: Futura Publishing Company, Inc.; 1997.
21. *Reller MD, Strickland MJ, Riehle-Colarusso T, Mahle WT, Correa A.* Prevalence of congenital heart defects in metropolitan Atlanta, 1998-2005. J Pediatr 2008; 153(6): 807−13.
22. *Cho SY, Oh JH, Lee JH, Lee JY, Lee SJ, Han JW,* et al.. Recent incidence of congenital heart disease in neonatal care unit of secondary medical center: a single center study. Korean J Pediatr 2012; 55(7): 232−7.
23. *Warnes CA, Liberthson R, Danielson GK, Dore A, Harris L, Hoffman JI,* et al. Task force 1: the changing profile of congenital heart disease in adult life. J Am Coll Cardiol 2001; 37(5): 1170−5.

24. *Kamphuis M, Ottenkamp J, Vliegen HW, Vogels T, Zwinderman KH, Kamphuis RP,* et al. Health related quality of life and health status in adult survivors with previously operated complex congenital heart disease. Heart 2002; 87(4): 356−62.
25. *Gersony WM, Hayes CJ, Driscoll DJ, Keane JF, Kidd L, O'Fallon WM*, et al. Second natural history study of congenital heart defects.Quality of life of patients with aortic stenosis, pulmonary stenosis, or ventricular septal defect. Circulation 1993; 87(2 Suppl): I52−65.
26. *Dickinson DF, Arnold R, Wilkinson JL*. Congenital heart disease among 160 480 liveborn children in Liverpool 1960 to 1969. Implications for surgical treatment. Br Heart J 1981; 46(1): 55−62.
27. *Hoffman JI*. Incidence of congenital heart disease: I. Postnatal incidence. Pediatr Cardiol 1995; 16(3): 103−13.
28. *Fixler DE, Pastor P, Chamberlin M, Sigman E, Eifler CW*. Trends in congenital heart disease in Dallas County births. 1971-1984. Circulation 1990; 81(1): 137−42. .
29. *Miranović V*. Frequencz and the results of treatment in children with congenital heart defects in Montenegro in a period 1995–2005 [dissertation]. Belgrade: University of Belgrade, Medical Faculty; 2007. (Serbian).

Received on September 17, 2014.
Accepted on December 12, 2014.
Online First May, 2015.

ORIGINAL ARTICLE

UDC: 616.255-089-06-08:612.78/.784
DOI: 10.2298/VSP141218144B

Voice analysis before and after vocal rehabilitation in patients following open surgery on vocal cords

Analiza glasa pre i posle vokalne rehabilitacije kod bolesnika posle otvorene operacije glasnica

Mila Bunijevac*†, Mirjana Petrović-Lazić†‡, Nadica Jovanović-Simić†, Mile Vuković†

***Public Health Hospital "St Vračevi", Bijeljina, Bosnia and Herzegovina; †Faculty for Special Education and Rehabilitation, University of Belgrade, Belgrade, Serbia; ‡ENT Clinic, Clinical Hospital Center "Zvezdara", Belgrade, Serbia**

Abstract

Background/Aim. The major role of larynx in speech, respiration and swallowing makes carcinomas of this region and their treatment very influential for patients' life quality. The aim of this study was to assess the importance of voice therapy in patients after open surgery on vocal cords. **Methods.** This study included 21 male patients and the control group of 19 subjects. The vowel (A) was recorded and analyzed for each examinee. All the patients were recorded twice: firstly, when they contacted the clinic and secondly, after a three-month vocal therapy, which was held twice *per* week on an outpatient basis. The voice analysis was carried out in the Ear, Nose and Throat (ENT) Clinic, Clinical Hospital Center "Zvezdara" in Belgrade. **Results.** The values of the acoustic parameters in the patients submitted to open surgery on the vocal cords before vocal rehabilitation and the control group subjects were significantly different in all specified parameters. These results suggest that the voice of the patients was damaged before vocal rehabilitation. The results of the acoustic parameters of the vowel (A) before and after vocal rehabilitation of the patients with open surgery on vocal cords were statistically significantly different. Among the parameters – Jitter (%), Shimmer (%) – the observed difference was highly statistically significant ($p < 0.01$). The voice turbulence index and the noise/harmonic ratio were also notably improved, and the observed difference was statistically significant ($p < 0.05$). The analysis of the tremor intensity index showed no significant improvement and the observed difference was not statistically significant ($p > 0.05$). **Conclusion.** There was a significant improvement of the acoustic parameters of the vowel (A) in the study subjects three months following vocal therapy. Only one out of five representative parameters showed no significant improvement.

Key words:
otorhinolaryngologic surgical procedures; laryngeal neoplasms; treatment outcome; voice quality; rehabilitation; speech production measurement.

Apstrakt

Uvod/Cilj. Glavna uloga larinksa – govor, disanje i žvakanje – čine da karcinomi tog regiona i njihovo lečenje veoma utiču na kvalitet života tih bolesnika. Cilj ove studije bio je da se proceni značaj vokalne terapije kod bolesnika posle otvorene operacije na glasnicama. **Metode.** Ovo ispitivanje obuhvatilo je 21 bolesnika muškog pola i kontrolnu grupu koju je činilo 19 ispitanika. Kod svih bolesnika, sniman je i analiziran intenzitet vokala (A). Svi bolesnici snimani su dva puta, prvi put kad su se javili u ambulantu i drugi put posle tromesečne vokalne terapije. Vokalna terapija sprovodila se ambulantno, dva puta sedmično. Analiza glasa izvršena je u ORL klinici Kliničko-bolničkog centra „Zvezdara" u Beogradu. **Rezultati.** Vrednosti akustičkih parametara kod bolesnika nakon otvorene operacije glasnica pre vokalne terapije i ispitanika kontrolne grupe značajno su se razlikovali u svim navedenim parametrima. Ovi rezultati ukazuju na to da je glas ispitanika pre vokalne terapije bio oštećen. Rezultati akustičkih parametara samoglasnika (A) pre i posle vokalne terapije kod bolesnika posle otvorene operacije na glasnicama statistički su se značajno razlikovali. Među parametrima Jitter (%) i Shimmer (%) razlika je bila visoko statistički značajna ($p < 0,01$). Indeks turbulencije glasa i odnos šum/harmonik takođe su se značajno poboljšali, a razlika je bila statistički značajna ($p < 0,05$). Analizom tremora kroz indeks intenziteta, nije došlo do značajnog poboljšanja, a posmatrana razlika nije bila statistički značajna ($p > 0,05$). **Zaključak.** Nakon tromesečne vokalne terapije došlo je do značajnog napretka akustičkih parametara intenziteta vokala (A) analiziranih ispitanika. Od pet analiziranih reprezentativnih parametara samo jedan nije imao značajan napredak.

Ključne reči:
hirurgija, otorinolaringološka, procedure; larinks, neoplazme; lečenje, ishod; glas, kvalitet; rehabilitacija; govor, produkcija, merenje.

Correspondence to: Mila Bunijevac, Public Health Hospital "St. Vračevi", Srpske Vojske 53, 76 300 Bijeljina, Republika Srpska, Bosnia and Herzegovina. E-mail: bunijevacmila@yahoo.com

Introduction

Partial laringectomy was introduced in order to alleviate a handicap associated with total laryngectomy. Partial laringectomy surgery removes only affected laryngeal parts preserving intact airway and providing notably less damage to vocal generator.

Cordectomy *via* thyrotomy is the oldest surgical procedure for the treatment of early glottic carcinoma [1].

Cordectomy can be performed by the following two methods depending on the indication: endoscopic laser cordectomy, and laryngofissure with cordectomy.

The major role of larynx in speech, respiration and swallowing makes carcinomas of this region and their treatment very influential for patients' life quality. Laryngeal carcinomas are second in frequency among all primary epithelial malignant tumors of head and neck and they make 2% of all human carcinomas.

Treatment of early stage glottic carcinoma, in the most cases, is successful for two reasons. Firstly, carcinoma of the true vocal cord gives an early symptom – hoarseness – that is relatively easy to be diagnosed. Secondly, the glottic region is insufficient in lymph drainage which provides spreading to regional lymph nodes extremely rare, less than 1% within stadium T1. All these factors enables high curability of glottic carcinomas in early stage [2].

The aim of this study was to assess the importance of voice therapy in patients after open surgery on vocal cords.

Methods

This research involved 21 male examinees and the control group of 19 subjects. The average age of the subjects was 48.9 ± 4.55 years. All the patients were recorded twice: firstly, when they contacted the Clinic and secondly, after a three-month vocal therapy, which was held twice *per* week on an outpatient basis. The examinees vocalized a vowel (A) in the most adequate height for them individually, afterwards the mean produced values were analyzed. Acoustic parameters of the vowel (A) of each examinee were analyzed in real time. Acoustic interpretation was performed in the ENT Clinic, Clinical Center "Zvezdara" in Belgrade. The success of vocal therapy was followed by a computerized laboratory for vocal analysis, model 4300, "Kay Elemetrics" company. This computerized laboratory provides unbiased voice analysis records and concurrently serves as a support for subjective assessment of voice. This study was conducted among the patients who had undergone open surgery. Five parameters of multidimensional voice analysis were studied: parametric signal (jitter percent %), noise parameters [noise/harmonic ratio (NHR)], voice turbulence index (VTI), tremor parameter [the frequency tremor (Fo) intensity index (FTRI %)].

In the paper the descriptive statistics methods were applied: measures of variability and tabulation. The analytical statistics methods were as follows: Student's *t*-test for parametric and Wilcoxon test of equivalent W-pairs for nonparametric data. Since the sample size was 21, the significance of differences in Wilcoxon test was determined by Zed-Z test, because the data were in normal distribution.

Results

This research involved 21 male examinees and the control group of 19 subjects. The average age of the subjects was 48.9 ± 4.55 years. The distribution of factors associated with glotis carcinoma is shown in Table 1. Most often the patients had all of the factors we tested, we labeled these factors as associated ones (87.8%). Analyzing them separtely smoking was the most common factor (76.20%), then work in air polluted enviroment (66.5%), and inflammatory processes (47.0%).

Table 1

Representation of factors associated with glotis carcinoma

Factors	Yes (%)	No (%)
Work in an area with air pollution	66.50	33.50
Smoking	76.20	23.80
Inflammatory processes	47.00	53.00
Associated factors	87.80	12.20

Analyzing the results, we firstly compared the values of acoustic parameters in the examinees before vocal rehabilitation and of the control group before vocal rehabilitation, too.

The obtained results are shown in Table 2. We noticed that values of all processed parameters were highly statistically different. This indicated that the patients' voice was damaged significantly. The obtained values of the parameters were

Table 2

Values of vocal parameters of the patients before vocal rehabilitation and the control group examinees

Vocal parameters	Patients with open surgery on vocal cords (n = 21)		Control group (n = 19)		Difference		*p*
	Mean value	SD	Mean value	SD	Absolute	%	
Jitter (%)	2.005	1.475	0.497	0.222	1.510	7.37	< 0.01
Shimmer (%)	5.646	2.520	1.844	0.437	3.810	67.32	< 0.01
NHR (db)	0.162	0.051	0.112	0.013	0.115	71.72	< 0.01
VTI	0.072	0.026	0.043	0.016	0.020	32.30	< 0.01
FTRI (%)	1.190	0.849	0.318	0.137	0.871	73.21	< 0.01

Jitter – variability of the fundamental frequency; Shimmer - variations in the amplitude of primary laryngeal tone; VTI – voice turbulence index; FTRI – frequency tremor intensity parameter; NHR – relationship of intraharmonic noise and harmonics; *p* – statistical significance.

following: Jitter(%): W = 480, Z = -4.360, $p < 0.01$; Shimmer (%): W = 466, Z = -4.153, $p < 0.01$; NHR (db): W = 458, Z = -4.262, $p < 0.01$; VTI: W = 460, Z = -4.498, $p < 0.01$; FTRI (%): W = 472, Z = -4.519, $p < 0.01$.

Table 3 shows the values of acoustic parameters before and after vocal rehabilitation. The results of acoustic parameters of the vowel (A) before and after vocal rehabilitation were significantly different. The observed difference for the parameter of short-term and long-term frequency disturbances- Jitter percent (%) and Shimmer percent (%) was statistically significant (W = 485, Z = -4.882, $p < 0.01$; and respectively) W = 452, Z = -4.756, $p < 0.01$, and respectively). VTI also notably improved, the observed difference was highly statistically significant (W = 129.1, Z = -2.154, $p < 0.05$). Analyzing the tremor through FTRI, no significant improvement was noticed. The observed difference was not statistically significant (W = 164, Z = -1.259, $p > 0.05$). The presence of noise in the analyzed signal – NHR, significantly changed after therapy. The observed difference was statistically significant (W = 129.1, Z =- 2.154, $p < 0.05$).

Table 4 shows the values of acoustic parameters in the examinees after vocal rehabilitation and in the control group subjects. The results showed that the values of the acoustic parameters in the patients after vocal rehabilitation and the value of acoustic parameters in the control group, except for FTRI (%) were not significantly different. The obtained values of parameters were as follows: Jitter (%) – W = 147, Z = -1.360, $p > 0.05$; Shimmer – W = 166, Z = -1.153, $p > 0.05$; NHR (db) – W = 158, Z = -1.262, $p > 0.05$.

Discussion

In this research the success of vocal therapy in the examinees was followed by multidimensional analysis of the vowel (A) vocal parameters. Many studies documented effects of vocal therapy in improving the acoustic parameters of voice [3–6]. Our results on distribution of predisposing factors for glotis carcinoma are congruent with the other authors' data [4, 7].

Numerous researches confirmed that normal and pathological voice differ in many parameters. Pathological voice is characterized by high values of frequency disturbances and amplitude disturbances. Our study affirms the same.

The differences between the values of the acoustic parameters before vocal rehabilitation and of the control group with all specified parameters were highly statistically significant. These results suggest that the voice of the examinees was considerably damaged before the beginning of vocal rehabilitation.

The results of the acoustic parameters of the vowel (A) before and after vocal rehabilitation were significantly different. The observed difference of two parameters – Jitter (%)

Table 3

Values of vocal parameters before and after vocal rehabilitation

Vocal parameters	Before vocal rehabilitation (mean value ± SD)	After vocal rehabilitation (mean value ± SD)	Difference		*p*
			Absolute	%	
Jitter (%)	1.991± 1.388	0.561 ± 0.250	1.423	72.59	< 0.01
Shimmer (%)	5.522 ± 5.456	1.990 ± 0.642	3.663	65.10	< 0.01
NHR (db)	0.157 ± 0.041	0.111 ± 0.008	0.045	29.10	< 0.05
VTI	0.071 ± 0.026	0.048 ± 0.013	0.016	23.66	< 0.05
FTRI (%)	1.202 ± 0.849	0.321 ± 0.155	0.868	72.91	> 0.05

Jitter – variability of the fundamental frequency; Shimmer – variations in the amplitude of primary laryngeal tone; VTI – voice turbulence index; FTRI – frequency tremor intensity parameter; NHR – relationship of intraharmonic noise and harmonics; *p* – statistical significance.

Table 4

Vocal parameters of the patients after vocal rehabilitation and the control group examinees

Vocal parameters	Patients after vocal rehabilitation (mean value ± SD)	Control group (mean value ± SD)	Difference		*p*
			Absolute	%	
Jitter (%)	0.561 ± 0.250	0.508 ± 0.167	0.052	9.42	> 0.05
Shimmer (%)	1.982 ± 0.642	1.844 ± 0.438	0.137	6.95	> 0.05
NHR (db)	1.396 ± 0.391	1.101 ± 0.364	0.294	21.11	> 0.05
VTI	0.111 ± 0.008	0.110 ± 0.007	0.001	0.88	> 0.05
FTRI (%)	0.321 ± 0.145	0.318 ± 0.147	0.002	0.92	< 0.05

Jitter – variability of the fundamental frequency; Shimmer – variations in the amplitude of primary laryngeal tone; VTI – voice turbulence index; FTRI – frequency tremor intensity parameter; NHR – relationship of intraharmonic noise and harmonics; *p* – statistical significance.

and Shimmer (%) was highly statistically significant ($p < 0.01$). VTI and NHR were also significantly improved, the observed difference was statistically significant ($p < 0.05$). Similar results were obtained by other authors [5, 7–10]. Analyzing tremor through FTRI, no significant improvement was observed and the observed difference was not statistically significant ($p > 0.05$). Examining available literature we did not find information on this parameter. A possible explanation for unsatisfying improvement of FTRI is the short duration of vocal rehabilitation. We can ascertain that the three-month vocal rehabilitation yielded good results and that it gave a great improvement of acoustic voice parameters, considering that only one of the analyzed parameters did not have a statistically significant improvement.

The gained results also showed that the values of acoustic parameters in the examinees after vocal rehabilitation and the values of acoustic parameters in the control group were not significantly different, except for one parameter – FTRI (%). Only with FTRI (%) the difference was statistically significant ($p < 0.05$). This was expected due to the abscence of improvement after vocal therapy.

Conclusion

The results of this research show that patients after open surgery on the vocal cords could repair their voice qualities by vocal therapy.

On the basis of the obtained results it could be concluded that a three-month vocal rehabilitation improves acoustic parameters of the vowel (A). Only one, out of five analyzed parameters showed no significant improvement. The computerized laboratory for vocal analysis, which was used in this study, provides visual, acoustic and aerodynamic information. This computerized laboratory provides unbiased voice analysis records and concurrently serves as a support for subjective assessment of voice. It could be used to confirm comparability. Pre-treatment and post-treatment data could be documented with a patient submitted to the program of vocal therapy. These data are helpful for the process of establishing the final diagnosis, as well as for the process of therapy. In this way the computerized laboratory for vocal analysis is very valuable both for patients and therapists.

R E F E R E N C E S

1. *de Diego J, Prim M, Verdaguer J, Pérez-Fernández E, Gavilán J.* Long-term results of open cordectomy for the treatment of T1a glottic laryngeal carcinoma. Auris Nasus Larynx 2009; 36(1): 53−6.
2. *Mikic A, Petrovic Z, Djukic V, Dimitrijevic M, Stankovic P, Pendjer I,* et al. Surgery treatment of laryngeal carcinoma T1. Acta Chir Iugoslav 2004; 51(1): 20−5. (Serbian)
3. *Deliyski D.* Acoustic model and evaluation of pathological voice production. In: Proceedings of the 3rd Conference on Speech Communication and Technology. 1993 Sept 21−23; Berlin, Germany: Eurospeech; 1993. p. 1969−72.
4. *Laver J, Hiller S, Beck JM.* Acoustic waveform perturbations and voice disorders. J Voice 1992; 6(2): 115−26.
5. *Petrović-Lazić M, Dobrota N, Jovanović-Simić N.* Multidimensional analysis of vowels in functional dysphonia's. In: World Congress of the International Association of Logopaedics and Phoniatrics (XXVII); Brisbane; 2007 August 6−9. Copenhagen: Tehnical University Denmark; 2007
6. *Jotic A, Stankovic P, Jesic S, Milovanovic J, Stojanovic M, Djukic V.* Voice quality after treatment of early glottic carcinoma. J Voice 2012; 26(3): 381−9.
7. *MacKenzie K, Millar A, Wilson J, Sellars C, Deary I.* Is voice therapy an effective treatment for dysphonia? A randomised controlled. Br Med J 2001; 323(7314): 658.
8. *Vilaseca I, Huerta P, Blanch JL, Fernández-Planas AM, Jiménez C,Bernal-Sprekelsen M.* Voice quality after CO2 laser cordectomy--what can we reallyexpect? Head Neck 2008; 30(1): 43−9.
9. *Galletti B, Freni F, Cammaroto G, Catalano N, Gangemi G, Galletti F.* Vocal outcome after CO2 laser cordectomy performed on patients affected by early glottic carcinoma. J Voice 2012; 26(6): 801−5.
10. *van Gogh CD, Verdonck-de LI, Langendijk JA, Kuik DJ, Mahieu HF.* Long-term efficacy of voice therapy in patients with voice problems after treatment of early glottic cancer. J Voice 2012; 26(3): 398−401.

Received on December 18, 2014.
Revised on January 20, 2015.
Accepted on January 26, 2015.
Online First December, 2015.

ORIGINAL ARTICLE

UDC: 613.86-057.875:[616.89-008.441+616.89-008.454
DOI: 10.2298/VSP141106143S

The prevalence and socioeconomic correlates of depressive and anxiety symptoms in a group of 1,940 Serbian university students

Prevalencija i uticaj socioekonomskih faktora na nastanak depresivnosti i anksioznosti na uzorku od 1 940 studenata u Srbiji

Ivana Simić-Vukomanović*, Goran Mihajlović[†‡], Sanja Kocić[*†], Nela Djonović[*†], Dragić Banković[§], VladimirVukomanović[†], Slavica Djukić-Dejanović[†‡]

***Institute of Public Health Kragujevac, Kragujevac, Serbia; [†]Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia; [‡]Department of Psychiatry, Clinical Center Kragujevac, Kragujevac, Serbia; [§]Faculty of Natural Sciences and Mathematics, University of Kragujevac, Kragujevac, Serbia**

Abstract

Background/Aim. Mental health of university students is under increasing concern worldwide, because they face challenges which predisposes them to depression and anxiety. The aim of this study was to identify demographic and socioeconomic variables associated with depressive and anxiety symptoms among university students. **Methods.** This cross-sectional study on 1,940 university students was performed using a questionnaire including demographic and socioeconomic variables, Beck Depression Inventory and Beck Anxiety Inventory. **Results.** The prevalence of depressive symptoms in students was 23.6%, while the prevalence of anxiety symptoms was 33.5%. The depressive symptoms were significantly related to the study year (p = 0.002), type of faculty (p = 0.014), satisfaction with college major choice (p < 0.001), satisfaction with grade point average (p < 0.001). Female students (odds ratio – OR = 1.791, 95% confidence interval – CI = 1.351–2.374), older students (OR = 1.110, 95% CI = 1.051-1.172), students who reported low family economic situation (OR = 2.091, 95% CI = 1.383–3.162), not owning the room (OR = 1.512, 95% CI = 1.103–2.074), dissatisfaction with graduate education (OR = 1.537, 95% CI = 1.165–2.027) were more likely to show depressive symptoms. The anxiety symptoms were significantly related to study year (p = 0.034), type of faculty (p < 0.001), family economic situation (p = 0.011), college residence (p = 0.001) satisfaction with the college major choice (p = 0.001), and satisfaction with graduate education (p < 0.001). Female students (OR = 1.901, 95% CI = 1.490–2.425), and students who reported parents high expectations of academic success (OR = 1.290, 95% CI = 1.022–1.630) were more likely to show anxiety symptoms. **Conclusion.** This is one of the largest study examining mental disorders in a sample of university students in Serbia. These findings underscore the importance of early detections of mental problems and prevention interventions in university students.

Key words:
depression; anxiety; students; signs and symptoms; prevalence; socioeconomic factors; serbia.

Apstrakt

Uvod/Cilj. Mentalno zdravlje studenata postaje sve više sfera interesovanja na globalnom nivou, jer sučeljavanje sa brojnim životnim situacijama koje nosi ovaj period života ubrzava nastanak depresivnih i anksioznih poremećaja. **Metode.** Istraživanje je sprovedeno kao epidemiološka studija preseka na uzorku od 1 940 studenata, korišćenjem upitnika koji je pored demografskih i socioekonomskih karakteristika obuhvatao Bekovu skalu za procenu depresivnosti i Bekovu skalu za procenu anksioznosti. **Rezultati.** Na ispitivanom uzorku, prevalencija depresivnih simptoma iznosila je 23,6% a prevalencija anksioznih simptoma 33,5%. Utvrđeno je da postoji statistički značajna povezanost nastanka depresivnih simptoma sa godinom studija (p = 0,002), vrstom fakulteta (p = 0,014), zadovoljstvom izborom fakulteta (p < 0,001) i zadovoljstvom prosečnom ocenom (p < 0,001). Regresionom analizom dobijeno je da su ženski pol (OR = 1,791, 95% CI = 1,351–2,374), stariji uzrast (OR = 1,110, 95% CI = 1,051–1,172), loš imovni status porodice (OR = 2,091, 95% CI = 1,383–3,162), neposedovanje svoje sobe (OR = 1,512, 95% CI = 1,103–2,074), nezadovoljstvo uslovima studiranja (OR = 1,537, 95% CI = 1,165–2,027) pokazatelji nastanka depresivnih simptoma. U pogledu anksioznih simptoma, utvrđena

Correspondence to: Ivana M. Simić Vukomanović, Institute of Public Health Kragujevac, Nikola Pašić 1, 34 000 Kragujevac, Serbia. E-mail: drivanasimic@gmail

je statistički značajna povezanost sa godinom studija (p = 0,034), vrstom fakulteta (p < 0,001), mestom stanovanja za vreme studiranja (p = 0,001), porodičnim imovnim statusom (p = 0,011), zadovoljstvom izbora fakulteta (p = 0,001) i zadovoljstvom uslovima studiranja (p < 0,001). Regresionom analizom dobijeno je da su ženski pol (OR = 1,901, 95% CI = 1,490–2,425) i visoka očekivanja roditelja uspeha na studijama (OR = 1,290, 95% CI = 1,022–1,630) prediktori nastanka anksioznih simptoma. **Zaključak.** Studija predstavlja jedno od najvećih istraživanja koje se bavi procenom mentalnog zdravlja populacije studenata na teritoriji Srbije. Dobijeni rezultati ukazuju na značaj ranog prepoznavanja problema iz oblasti mentalnog zdravlja u cilju pripreme preventivnih programa.

Ključne reči:
depresija; anksioznost; studenti; znaci i simptomi; prevalenca; socioekonomski faktori; srbija.

Introduction

Mental health problems are a major public health concern due to their high prevalence rates, difficult treatment, and often chronic course [1]. In addition, the unrecognized burden of depression and anxiety became undeniably evident in developed and developing countries until the year 2000 [2]. In the European region, mental disorders, including anxiety and depression, are the second largest contributor to the burden of disease (measured using disability-adjusted life years − DALYs) and the most important cause of disability [3]. By the year 2020, if current trends for demographic and epidemiological transition continue, burden of depression will become the second leading cause of DALYs lost [4]. Anxiety disorders also rank among the twenty conditions contributing the largest global share of years lived with disability (YLDs) [5]. Furthermore, most lifetime mental disorders have their first onset during the typical university age [6, 7], making depression a particularly salient problem area for student population [8]. Worldwide estimation of current depression prevalence range upwards from 8% [9] to as high as 85% among university students [10]. Regarding anxiety symptoms, the prevalence will range from 8% [9] to 47.7% [10]. Generally, the prevalence seems to be increasing. In the United States of America, the National College Health Assessment reported that 1 in 3 undergraduates had at least one episode in the previous year of "feeling so depressed it was difficult to function" [7]. With symptoms of nearly three-fourths of all lifetime diagnosable mental health disorders it is critical to identify these disorders as early in life as possible [11].

Studies show that female students had almost two times higher level of depression compared to their male counterparts [12]. Socioeconomic parameters that are connected with the prevalence of depression are low incomes and financial problems (lower socioeconomical status), lower education level, bad living conditions and urban life style [13]. Higher anxiety level in female (aged 20–30 years) shows no differences regarding religion and socioeconomical status [14]. Other potential stressors for depression and anxiety can be: transition to university life, acclimating to a new environment, establishing new social networks, meeting their personal goals [15], academic factors (year of study, area of study) [16], academic overload and demands, financial pressures, pressure to succeed [17], separation from their usual support network [15, 18]. On the other hand, sometimes there is a high level of stigmatization associated with mental illness [19]. Previously obtained data in Serbia showed that about 1/3 of high school and university students population manifested signs of psychological distress and has mental problems [20]. Moreover, mild depression was reported to be six times more prevalent than severe depression and it was more prevalent in the 20–24 age groups. This could be considered as a kind of maladaptive behavior [21].

From the public health perspectives, early detection of mental health problems is essential, especially in young adults, in order to conduct appropriate screening and intervention programs [22, 23] and to improve the longer-term prognosis related to future risk of depression [24].

The general applicability of published results concerning student's mental health is limited. Most studies are based on samples that are not representative of the general student population, due to confinedment to a single faculty, to the specified years of study to one academic field or are selected using non-probabilistic methods [16]. After considering all these factors, we planed to estimate the prevalence of depressive and anxiety symptoms and to examine the specific demographic, socioeconomic correlates of depressive and anxiety symptoms.

Methods

Study design and participants

The study was a cross-sectional survey of students attending University of Kragujevac, Serbia, in the year 2013/2014. University of Kragujevac, with its twelve faculties, is a state-owned university in Central Serbia. Six of its faculties are located in Kragujevac, while the other six faculties are located in five towns of Central Serbia, thus covering the area with more than 2,500,000 inhabitants. All the twelve faculties were selected for the survey: Faculty of Agronomy, Faculty of Economics, Faculty of Engineering, Faculty of Mechanical and Civil Engineering, Faculty of Medical Sciences, Faculty of Education, Faculty of Law, Faculty of Natural Sciences and Mathematics, Faculty of Technical Sciences, Teachers Training Faculty, Faculty of Philology and Arts, Faculty of Hotel Management and Tourism. The students were randomly sampled from every study year of each faculty, in proportion to the size of the faculty in relation to the total number of students in University. We randomly selected 1,940 students from the total of 18,123 students at the University of Kragujevac. The students were sorted out from the university students data base according to previously generated random order (random computer function).

Procedure

A self administered anonymous questionnaire which comprised of demographic and socioeconomic variables, Beck Depression Inventory (BDI-IA) and Beck Anxiety Inventory (BAI) were used. Ethical approval was obtained from the Faculty of Medical Sciences Ethical Committee. Participation was completely voluntary, with no economic or other motivation. Informed consent was obtained, and confidentiality of the responses was assured. The study was conducted in the participants own classrooms by the leading researcher. Those who were absent during the distribution of questionnaires were excluded. The research was completed within 35 weeks.

Instruments

A self-assessment questionnaire with detailed subdomain questions was used to determine variables. Symptoms of depression were evaluated through the scale BDI-IA. This scale was developed in the 1960's and is one of the most widely used instruments for measuring the severity of depression, with the focus on behavioral and cognitive aspects of these disorders [25]. It was designed to document a variety of depressive symptoms the individual experienced over the preceding week. It consists of 21 items, each answer being scored on a scale ranging from 0 to 3. The total score has a minimum of 0 and a maximum of 63. The rating scale was as follows: 0 to 9 – no symptoms, 10 to 15 – mild mood change or mild depression state; 16 to19 – mild to moderate depression, 20 to 29 – moderate depression and 30 to 63 – severe or clinical depression The internal consistency for the BDI-IA was good, with average alpha coefficient of 0.81 for non psychiatric samples and with highly intercorrelated items [26].

Symptoms of anxiety were evaluated through the BAI scale, a short list describing 21 anxiety symptoms which bothered them in the past week. The scale consists of 21 items, each answer being scored on a scale ranging from 0 to 3. The total score has a minimum of 0 and a maximum of 63. A total score of 0 to 7 is interpreted as a "minimal" level of anxiety, 8 to 15 as a "mild" level of anxiety, 16 to 25 as a "moderate" level of anxiety, and 26 to 63 as a "severe" level of anxiety [27].

Statistical analysis and assessment

Data analysis was carried out using IBM Statistical Package for the Social Sciences (SPSS) software version 19.0. Data cleaning was done to detect any missing values, coding error or any illogical data values. The qualitative variables (demographic and socioeconomic) were presented with the numbers and as a percentage. The continuous variables (depression, anxiety and symptoms scores), were presented as means and standard deviation (SD). Descriptive statistics for all sociodemographic characteristics, depressive and anxiety symptoms of the participants were calculated, expressed as appropriately in frequencies, mean values and standard deviation. Student's *t*-test, Fisher's exact test χ^2 Pearson and Spearman correlations were all used to look for any existing association between demographic and socioeconomic characteristics, and anxiety and depression. All tests were 2-tailed, and the level of significance was set at $p < 0.05$.We conducted univariate and multivariate logistic regression analysis to study associations between depressive, anxiety symptoms and potential risk factors. The results are reported as odds ratios (OR) with 95% confidence intervals (CI).

Results

From 1,968 distributed questionnaires, a total of 1,940 students completed questionnaire during the survey with the response rate of 98.6%. The mean age of the participating students was 21.04 (SD = ± 2.23) years with the range of 18–57 years. The demographic and socioeconomic characteristics of the sample are summarized in Table 1.

Regarding depressive symptoms, the mean BDI-IA score was 6.12 (SD = ± 6.4), with the range between 0 and 63. Further analysis indicated that 15.4% of the students had the score between 10 and 15 (mild depression state), 4.2% the score between 16 and 19 mild to moderate depression), 2.9% the score between 20 and 29 (moderate depression), 1.1% the score between 30 and 63 (severe depression). The mean BAI score for anxiety symptoms was 6.88 (SD = ±7.3), with the range between 0 and 63. About 22.7% of the respondents had a score between 8 and 15 (mild anxiety), 7.4% the score between 16 and 25 (moderate anxiety), 3.4% the score between 26 and 63 (severe anxiety).

The depressive symptoms were significantly related to the study year ($p = 0.002$), the type of faculty ($p = 0.014$), satisfaction with the college major choice ($p < 0.001$), and satisfaction with grade point average ($p < 0.001$).

We found no significant association of depressive symptoms with father's educational level ($p = 0.815$), father's employment ($p = 0.669$), mother's educational level ($p = 0.969$), mother's employment, ($p = 0.393$), residence ($p = 0.928$) marital status, ($p = 0.510$) having children, ($p = 0.825$), college residence ($p = 0.097$), parent's high expectations of academic success ($p = 0.069$) and professors high expectations of academic success ($p = 0.158$). Association of depressive symptoms with potential risk factors is summarized in Table 2.

Analyses of logistic regression model indicated that the possibility of having depressive symptoms was significantly higher in students who were female (OR = 1.791; 95%, CI, 1.351–2.374) who were older (OR = 1.110; 95% CI, 1.051–1.172), had a low family economic situation (OR = 2.091; 95% CI, 1.383–3.162), had dissatisfaction with graduate education (OR = 1.537; 95% CI, 1.165–2.027) and students who did not have their own room (OR = 1.512; 95% CI, 1.103–2.074). Logistic regression model on depressive symptoms is shown in Table 3.

The anxiety symptoms were significantly related to study year ($p = 0.034$), type of faculty ($p < 0.001$), family economic situation ($p = 0.011$), college residence ($p = 0.001$), satisfaction with the college major choice ($p = 0.001$), and satisfaction with graduate education ($p < 0.001$).

No statistically significant relationship was found between anxiety symptoms and age ($p = 0.096$), father's

Table 1

Demographic and socioeconomic factors of the sample of university students (n = 1,940)

Variable	n	(%)
Gender (total number)	1,931	
male	672	34.8
female	1,259	65.2
Age (year), $\bar{x} \pm SD$	21.04 ± 2.23	
Study year (total number)	1,931	
1	577	29.9
2	519	26.9
3	385	19.9
4	300	15.5
5	67	3.5
6	83	4.3
Father's educational level (total number)	1,875	
uneducated	8	0.4
primary school	111	5.9
high school	1,335	71.2
university	421	22.5
Father's employment, (total number)	1,853	
yes	1,213	65.5
no	640	34.5
Mother's educational level (total number)	1,903	
uneducated	5	0.2
primary school	158	8.3
high school	1,324	69.6
university	416	21.9
Mother's employment (total number)	1,893	
yes	1,055	55.7
no	838	44.3
Residence (total number)	1,925	
urban	1,067	55.4
semi-urban	379	19.7
rural	479	24.9
Marital status (total number)	1,922	
never married	1,872	97.3
married	42	2.2
separated	3	0.2
widowed	5	0.3
Having children (total number)	1,924	
yes	54	2.8
no	1,870	97.2
Owning the room (total number)	1,908	
yes	327	17.1
no	1,581	82.9
Family economic situation (total number)	(n = 1,916)	
very good	126	6.6
good	642	33.5
moderate	982	51.3
poor	145	7.6
very poor	21	1
College residence (total number)	(n = 1,841)	
campus	218	11.8
other	1,623	88.2
Satisfaction with college major choice (total number)	1,920	
very	613	31.9
mostly	1,118	58.2
not particular	154	8
not at all	35	1.9
Satisfaction with graduate education (total number)	1,910	
yes	1,306	68.4
no	604	31.6
Satisfaction with grade point average (total number)	1,710	
yes	976	57.1
no	734	42.9
Parents high expectations of academic success (total number)	1,910	
I completely agree	161	8.4
I agree	484	25.3
I don't know	311	16.3
I don't agree	697	36.5
I absolutely disagree	257	13.5
Professors high expectations of academic success (total number)	1,880	
I completely agree	124	6.6
I agree	322	17.1
I don't know	796	42.3
I don't agree	477	25.4
I absolutely disagree	161	8.6

Table 2

Association of depressive symptoms with potential risk factors

Variable	Depressive symptoms (% of patients)			χ^2	DF	*p*
	none	mild	moderate & severe			
Gender						
male	82.0	10.9	7.1	17.71	2	< 0.001
female	73.4	17.8	8.7			
Age				25.138	2	< 0.001
Study year						
1	81.5	10.1	8.4	27.526	10	0.002
2	72.8	17.0	10.1			
3	75.4	18.0	6.6			
4	76.0	16.7	7.3			
5	77.4	11.3	11.3			
6	68.7	26.5	4.8			
Faculties				39.041	22	0.014
Owning the room						
yes	78.0	14.7	7.4	13.073	2	< 0.001
no	69.0	18.6	12.4			
Family economic situation						
very good	86.5	7.2	6.3	44.667	8	< 0.001
good	81.3	12.8	6.0			
moderate	74.8	16.1	9.1			
poor	59.8	26.5	13.6			
very poor	50.0	38.9	11.1			
Satisfaction with college major choice						
very	83.4	10.8	5.8	49.772	6	< 0.001
mostly	75.6	16.5	7.9			
not particular	59.3	24.3	16.4			
not at all	60.0	16.7	23.3			
Satisfaction with graduate education						
yes	80.8	12.6	6.6	38.257	4	< 0.001
no	67.3	21.5	11.1			
Satisfaction with grade point average						
yes	80.2	13.7	6.1	28.194	2	< 0.001
no	68.9	19.5	11.6			

DF – degrees of freedom.

Table 3

Logistic regression model on depressive symptoms

Variables	B	*p*	OR	OR (95% CI)	
Gender	0.583	0.000	1.791	1.351	2.374
Age	0.104	0.000	1.110	1.051	1.172
Low family economic situation	0.738	0.000	2.091	1.383	3.162
Owning the room	0.414	0.010	1.512	1.103	2.074
Dissatisfaction with graduate education	0.430	0.002	1.537	1.165	2.027

OR – odds ratio; CI – confidence interval.

educational level (p = 0.371), father's employment (p = 0.491), mother's educational level (p = 0.564), mother's employment (p = 0.933), residence (p = 0.677), marital status (p = 0.493), having children (p = 0.398), owning a room (p = 0.051), satisfaction with grade point average (p = 0.196), and professors high expectations of academic success (p = 0.113). Association of anxiety symptoms with potential risk factors is summarized in Table 4.

The possibility of having anxiety symptoms was significantly higher in students who were female (OR = 1.901; 95% CI, 1.490–2.425); and had parents with high expectations about academic success (OR = 1.290; 95% CI, 1.022–1.630). Logistic regression model on anxiety symptoms is shown in Table 5.

Discussion

The present study is one of the largest epidemiological studies, regarding mental health status among university students, in this region.

The primary objective of this study was to investigate the prevalence of depressive and anxiety symptoms in university students. We found that the prevalence of depressive symptoms was 23.6%, while the prevalence of anxiety symptoms was 33.5%. A significantly increased rate of depression in college students, previously reported from the U.S. and Western Europe studies, confirmed previous concerns about global growth [28], especially which includes deficits in cognitive, emotional and physical development [29]. Although,

Table 4

Association of anxiety symptoms with potential risk factors

Variable	Anxiety symptoms (% of patients)			x^2	DF	*p*
	none	mild	moderate & severe			
Gender						
male	76.6%	15.4%	8.0%	42.992	2	< 0.001
female	73.4%	17.8%	8.7%			
Study year						
1	71.6%	20.3%	8.1%	19.527	10	0.034
2	63.7%	24.4%	11.9%			
3	60.5%	24.6%	15.0%			
4	66.7%	22.2%	11.1%			
5	71.9%	19.3%	8.8%			
6	68.3%	25.6%	6.1%			
Faculties				53.365	22	< 0.001
Family economic situation						
very good	64.5%	20.6%	15.0%	19.799	8	0.011
good	70.2%	20.7%	9.1%			
moderate	65.0%	24.5%	10.5%			
poor	59.4%	24.2%	16.4%			
very poor	55.0%	15.0%	30.0%			
College residence						
campus	67.7%	21.3%	11.0%	13.637	2	0.001
other	56.4%	32.7%	10.9%			
Satisfaction with college major choice						
very	70.7%	20.7%	8.6%	22.43	6	0.001
mostly	66.0%	22.6%	11.4%			
not particular	53.2%	34.0%	12.8%			
not at all	60.0%	16.7%	23.3%			
Satisfaction with graduate education						
yes	69.3%	21.6%	9.1%	24.909	4	< 0.001
no	60.2%	25.3%	14.4%			
Parents high expectations of academic success						
I completely agree	56.1%	25.9%	18.0%	17.365	8	0.027
I agree	63.2%	24.3%	12.5%			
I don't know	65.4%	24.3%	10.4%			
I don't agree	70.5%	20.7%	8.7%			
I absolutelly disagree	67.2%	22.1%	10.6%			

DF – degrees of freedom.

Table 5

Logistic regression model on anxiety symptoms

Variables	B	*p*	OR	OR (95% CI)	
Gender	0.642	0.000	1.901	1.490	2.425
Parents high expectations of academic success	0.255	0.032	1.290	1.022	1.630

OR – odds ratio.

our findings for the prevalence of depressive and anxiety symptoms are higher than the results of a large American study (17.3% and 9.8%, respectively) [30], and Australian study (8% and 13% respectively) [9], they are similar with some European studies, especially about the prevalence of depressive symptoms (23% in Germany, 27.1% in Turkey) [18, 31]. Interestingly, other European studies reported higher occurrence of depressive symptoms (34% in Poland, 39% in Bulgaria, 52.4% in Greece) 15, while the lowest prevalence has been reported in the students in Switzerland (10.2%) [32]. All these variations could be explained by cultural differences, demographic and socioeconomic situation. One of the few studies from the former republics of Yugoslavia showed lower prevalence of depressive symptoms than our study (Croatia 9.4% [33], FYR Macedonia 10.4% 34) but higher for anxiety symptoms (FYR Macedonia 65.5% [34]). It should, however, be noted that these discrepancies may be due to the small sample sizes. No closely related research studies, on the prevalence of depressive and anxiety symptoms, have been previously conducted among university students in Serbia, thus there are no data available for comparison.

The second objective of this study was to examine the demographic, socioeconomic correlates of depressive and anxiety symptoms, and the results are summarized in Tables 2 and 4. The present study reported statistically significant differences in depressive symptoms by gender, with a higher prevalence among female student. Using regression analysis we found that female students were 79.1% likely to get dep-

ressive symptoms, while 90.1% were more likely to suffer from anxiety symptoms, compared to male students. This is consistent with the majority of the studies regarding depressive symptoms [15, 18, 30], while a number of studies have found either no differences [6, 7, 31] or the opposite pattern [17]. In addition, our results are consistent with higher rates of anxiety symptoms among females found by other researchers, although no significant association existed for depressive symptoms [35], [36]. One of the explanations may be because females are more likely to report concern, stress and feeling of lack of competence, different sociodemographic background or even including factors related to gender role and potentially stressful transforming events.

In our study depressive symptoms increased with increasing age, about 11% for every year of life. Tendencies showed significant increases from early adolescence, peaks in late adolescence (16–18 years) and decreases towards older ages [18, 37]. Potentially stressful events are presumed to elicit mental health problems, such as employment, economic situation, graduation and marriage pressures [6, 30, 38]. On the contrary, some other studies have failed to find this association [39].

This study shows that students with lower socioeconomic background had a statistically significantly higher risk of depressive and anxiety symptoms. Using regression analysis, we also observed that students which reported low socioeconomic situation substantially have 109.1% the likelihood to develop depressive symptoms compared with those in the highest socioeconomic group. The inverse relationship between socioeconomic status and mental health problems is well established in general population samples [6, 30, 35]. Additionally, a meta-analysis in different European countries found unambiguous evidence that financial struggles had higher odds for depression [18] and anxiety, as well [22].

Furthermore, our study concludes that there is a significant difference between the mean depression scores and not having own room, with a higher likelihood of depressive symptoms up to 51.2%. There were almost significant interactions regarding the student's anxiety symptoms. More than three persons per room are related to psychiatric illness [40] although some studies findings are contrary [39]. These findings might be correlated with sociological and culturological differences.

Another important aspect of our study, regarding subclinical depressive symptoms (usually corresponds to mild depressive episodes according to ICD-10 classification), revealed that it is high in such populations (15.4%). Studies from other countries show a wide variety of rates ranging between 10% and 44% [6, 41–44]. In our culture there is still no awareness that depression and anxiety are disorders of youth. Because of that, mild depression and anxiety sometimes are not diagnosed and treated by health professionals. This underlines the importance for establishing proper screening tools for early identification and treatment of subclinical forms of depression.

Some stressful life events like dissatisfaction with the college major choice and dissatisfaction with graduate education was significant associated with depressive and anxiety symptoms. Our study indicates that students who are dissatisfied with graduate education were 53.7% more likely to have depressive symptoms. Students exposed to heavy academic workloads, strong examination criteria and being overburdened with test schedule, contributed to many of unhealthy behaviors [44, 45] and were significantly associated with anxiety 30 and depressive symptoms [6, 31].

Consequently, the present study also revealed association between low overall success in grade point average and depressive symptoms, but no significant association with anxiety symptoms. These coincidence have been reported by other authors [46], while others failed to find this correlation [37].

Another important aspect of our study is statistically significant correlations of pressure for success and depressive and anxiety symptoms especially when they are not able to meet the expectations of their parents. The observed association signifies that students were 29.0% more likely to have anxiety symptoms. Pressure to perform well academically, parental expectations and criticism is a strong predisposing factor for depression [47]. These findings suggest that relationship with parents have a substantial causal relation with the depressive and anxiety symptoms, especially during this delicate period in their life.

Consistent with findings from similar studies [22, 31] we also found a significant correlation between the study year and higher level of depressive and anxiety symptoms. In addition, we found that the sixth year students had the highest average BDI-IA scores, compared with the first year students. Next, the highest average BAI scores had the third year students, in regard to senior students. Senior students had higher depression scores compared with freshmen because postgraduate students worry about employment and future perspective and these stresses could be risk factors for depressive and anxiety symptoms [6, 22, 37]. The severity of the employment situation in Serbia, aggravates the employment pressure of college graduate. The Statistical Office of the Republic of Serbia published that the unemployment rate for youth aged 15–24 is reaching nearly 50% [48] and that situation might be potential risk factor for mental disorders. The prevalence of anxiety and depressive symptoms were significantly related to the type of faculty. We also found that the students who were studying Faculty of Mechanical and Civil Engineering had the highest depression score (38.4%). Regarding anxiety, Faculty of Education (54.8%), Faculty of Hotel Management and Tourism (54.8%), Teachers Training Faculty (48.6%), had higher anxiety scores. Students who were studying Faculty of Engineering had the lowest depression and anxiety scores (12% and 24.1% respectively). Several studies have been conducted with various and controversial results [49–51] due to methodological issues that limit interpretation, different measure instruments and different settings and cultures.

The primary limitation of the study is its cross-sectional design, which does not permit inferences about possible causal relations between the explanatory variables and disorders of interest. It was not possible to assess the test–retest reliability of BDI/BAI in this sample as the survey was anonymous. Another limitation was the self-reported nature of this study. Finally, our sample comprised a group of students in just one university of Serbia, which may limit generalization of the results through the other universities. According to the Strategy 2020 by the World

Health Organization [52] strengthening mental health promotion programmes is highly relevant.

Conclusion

These results demonstrate that the high rates of depressive and anxiety symptoms among university students are related to academic, nonacademic and cultural backgrounds. The last several years have provided data that highlight a neglected public health problem in institutes of higher education. The importance of early identification, especially the minor signs of depression, could prevent or reduce its severity and chronicity. From a public health perspective, onset and development of mental illness in students is a potentially critical area for intervention programs. A particular challenge is to promote the early diagnosis of depression by initiating community-based intervention programmes and to reduce the stigma of mood disorders. Such efforts hold substantial promise for the development of interventions that may have a positive impact on the health and well-being of college students.

Conflict of interest

All the authors declare that they have no conflicts of interest.

REFERENCES

1. *Melo-Carrillo A, Van Oudenhove L, Lopez-Avila A.* Depressive symptoms among Mexican medical students: high prevalence and the effect of a group psychoeducation intervention. J Affect Disord 2012; 136(3): 1098−103.
2. *Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C,* et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380(9859): 2197−223.
3. *Wittchen HU, Jacobi F, Rehm J, Gustavsson A, Svensson M, Jönsson B,* et al. The size and burden of mental disorders and other disorders of the brain in Europe 2010. Eur Neuropsychopharmacol 2011; 21(9): 655−79.
4. *Sayers J.* World Health Report 2001 - Mental Health: New Understanding, New Hope. Bull World Health Organ 2001; 79(11): 1085.
5. *Becker AE, Kleinman A.* Mental Health and the Global Agenda. N Engl J Med 2013; 369(1): 66−73.
6. *Chen L, Wang L, Qiu XH, Yang XX, Qiao ZX, Yang YJ,* et al. Depression among Chinese university students: prevalence and socio-demographic correlates. PLoS One 2013; 8(3): e58379.
7. *Sarokhani D, Delpisheh A, Veisani Y, Sarokhani MT, Manesh RE, Sayehmiri K.* Prevalence of Depression among University Students: A Systematic Review and Meta-Analysis Study. Depress Res Treat 2013; 2013: 373857.
8. *Reavley N, Jorm AF.* Prevention and early intervention to improve mental health in higher education students: a review. Early Interv Psychiatry 2010; 4(2): 132−42.
9. *Said D, Kypri K, Bowman J.* Risk factors for mental disorder among university students in Australia: findings from a web-based cross-sectional survey. Soc Psychiatry Psychiatr Epidemiol 2013; 48(6): 935−44.
10. *Ibrahim AK, Kelly SJ, Adams CE, Glazebrook C.* A systematic review of studies of depression prevalence in university students. J Psychiatr Res 2013; 47(3): 391−400.
11. *Kessler RC, Berglund P, Demler O, Jin R, Merikangas KR, Walters EE.* Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. Arch Gen Psychiatry 2005; 62(6): 593−602.
12. *Waraich P, Goldner EM, Somers JM, Hsu L.* Prevalence and incidence studies of mood disorders: a systematic review of the literature. Can J Psychiatry 2004; 49(2): 124−38.
13. *Singleton N, Bumpstead R, O`Brien M, Lee A, Meltzer H.* Psychiatric morbidity among adults living in private households, 2000. Int Rev Psychiatry 2003; 15(1−2): 65−73.
14. *Adamovic V, Erić Lj, Kaličanin P, Starčević V.* Psycho-Neuroses. Belgrade: School of Medicine; 2000. (Serbian)
15. *Sokratous S, Merkouris A, Middleton N, Karanikola M.* The association between stressful life events and depressive symptoms among Cypriot university students: a cross-sectional descriptive correlational study. BMC Public Health 2013; 13(1): 1121.
16. *Saïas T, Roscoät ED, Véron L, Guignard R, Richard J, Legleye S,* et al. Psychological distress in French college students: demographic, economic and social stressors. Results from the 2010 National Health Barometer. BMC Public Health 2014; 14(1): 256.
17. *Sidana S, Kishore J, Ghosh V, Gulati D, Jiloha R, Anand T.* Prevalence of depression in students of a medical college in New Delhi: A cross-sectional study. Australas Med J 2012; 5(5): 247−50.
18. *Mikolajczyk RT, Maxwell AE, Naydenova V, Meier S, el Ansari W.* Depressive symptoms and perceived burdens related to being a student: Survey in three European countries. Clin Pract Epidemiol Ment Health 2008; 4:19.
19. *Royal College of Psychiatrists.* College Report CR166: Mental health of students in higher education. London (UK): Royal College of Psychiatrists; 2011.
20. *Expert Group on Youth Development and Health of the Ministry of Health of Serbia.* Strategy for youth development and health in the Republic of Serbia. Belgrade: Ministry of Health of the Republic of Serbia; 2006.
21. *Talaei A, Fayyaz MR, Ardani AR.* Depression and Its Correlati on With Self-esteem and Social Support Among Iranian University Students. Iran J Psychiatry 2009; 4: 17−22.
22. *Shamsuddin K, Fadzil F, Ismail WS, Shah SA, Omar K, Muhammad NA,* et al. Correlates of depression, anxiety and stress among Malaysian university students. Asian J Psychiatr 2013; 6(4): 318−23.
23. *Mirkovic M, Simic S, Trajkovic G.* Assessment of mental health in adults of the northern part of the city of Kosovska Mitrovica. Vojnosanit Pregl 2012; 69(9): 747−52.
24. *Reyes-Rodríguez ML, Rivera-Medina CL, Cámara-Fuentes L, Suárez-Torres A, Bernal G.* Depression symptoms and stressful life events among college students in Puerto Rico. J Affect Disord 2013; 145(3): 324−30.
25. *Beck AT, Steer RA.* Manual for the Beck Depression Inventory. San Antonio, TX: Psychological Corporation; 1993.
26. *Ignjatović-Ristić D, Hinić D, Jović J.* Evaluation of the Beck Depression Inventory in a nonclinical student sample. West Indian Med J 2012; 61(5): 489−93.
27. *Beck AT, Steer RA.* Beck Anxiety Inventory (BAI) Manual. Oxford (UK): Pearson; 1990.
28. *Steptoe A, Tsuda A, Tanaka Y, Wardle J.* Depressive symptoms, socio-economic background, sense of control, and cultural fac-

tors in university students from 23 countries. Int J Behav Med 2007; 14(2): 97−107.
29. *Marmot MG, Bell R.* How will the financial crisis affect health. BMJ 2009; 338: b1314.
30. *Eisenberg D, Hunt J, Speer N.* Mental health in American colleges and universities: variation across student subgroups and across campuses. Int J Behav Med 2013; 14(2): 97−107.
31. *Bayram N, Bilgel N.* The prevalence and socio-demographic correlations of depression, anxiety and stress among a group of university students. Soc Psychiatry Psychiatr Epidemiol 2008; 43(8): 667−72.
32. *Christensson A, Vaez M, Dickman PW, Runeson B.* Self-reported depression in first-year nursing students in relation to socio-demographic and educational factors: a nationwide cross-sectional study in Sweden. Soc Psychiatry Psychiatr Epidemiol 2010; 46(4): 299−310.
33. *Yang F, Meng H, Chen H, Xu X, Liu Z, Luo A,* et al. Influencing factors of mental health of medical students in China. J Huazhong Univ Sci Technolog Med Sci 2014; 34(3): 443−9.
34. *Uglesić B, Lasić D, Zuljan-Cvitanović M, Buković D, Karelović D, Delić-Brkljacić D,* et al. Prevalence of depressive symptoms among college students and the influence of sport activity. Coll Antropol 2014; 38(1): 235−9.
35. *Mancevska S, Bozinovska L, Tecce J, Pluncevik-Gligoroska J, Sivevska-Smilevska E.* Depression, anxiety and substance use in medical students in the Republic of Macedonia. Bratisl Lek Listy 2008; 109(12): 568−72.
36. *Eisenberg D, Gollust SE, Golberstein E, Hefner JL.* Prevalence and correlates of depression, anxiety, and suicidality among university students. Am J Orthopsychiatry 2007; 77(4): 534−42.
37. *Bostanci M, Ozdel O, Oguzhanoglu NK, Ozdel L, Ergin A, Ergin N,* et al. Depressive symptomatology among university students in Denizli, Turkey: prevalence and sociodemographic correlates. Croat Med J 2005; 46(1): 96−100.
38. *Alvi T, Assad F, Ramzan M, Khan FA.* Depression, anxiety and their associated factors among medical students. J Coll Physicians Surg Pak 2010; 20(2): 122−6.
39. *Ibrahim AK, Kelly SJ, Glazebrook C.* Analysis of an Egyptian study on the socioeconomic distribution of depressive symptoms among undergraduates. Soc Psychiatry Psychiatr Epidemiol 2012; 47(6): 927−37.
40. *Ghanem M, Gadallah M, Meky FA, Mourad S, El-Kholy G.* National survey of prevalence of mental disorders in Egypt: preliminary survey. East Mediterr Health J 2009; 15(1): 65−75.
41. *Al-Busaidi Z, Bhargava K, Al-Ismaily A, Al-Lawati H, Al-Kindi R, Al-Shafaee M,* et al. Prevalence of Depressive Symptoms among University Students in Oman. Oman Med J2011; 26(4): 235−9.
42. *Garlow SJ, Rosenberg J, Moore DJ, Haas AP, Koestner B, Hendin H,* et al. Depression, desperation, and suicidal ideation in college students: results from the American Foundation for Suicide Prevention College Screening Project at Emory University. Depress Anxiety 2008; 25(6): 482−8.
43. *Ibrahim MB, Abdelreheem MH.* Prevalence of anxiety and depression among medical and pharmaceutical students in Alexandria University. Alexandria J Med 2014. (In Press)
44. *Goebert D, Thompson D, Takeshita J, Beach C, Bryson P, Ephgrave K,* et al. Depressive symptoms in medical students and residents: a multischool study. Acad Med 2009; 84(2): 236−41.
45. *Baldassin S, Silva N, de Toledo FA, Castaldelli-Maia JM, Bhugra D, Nogueira-Martins MC,* et al. Depression in medical students: cluster symptoms and management. J Affect Disord 2013; 150(1): 110−4.
46. *Field T, Diego M, Sanders C.* Adolescent depression and risk factors. Adolescence 2001; 36(143): 491−8.
47. *Bhasin SK, Sharma R, Saini NK.* Depression, anxiety and stress among adolescent students belonging to affluent families: a school-based study. Indian J Pediatr 2010; 77(2): 161−5.
48. Abbreviated Country Development Cooperation Strategy, Fiscal Year 2013-2017. Belgrade: USAID; 2013.
49. *Dyrbye LN, Thomas MR, Shanafelt TD.* Systematic review of depression, anxiety, and other indicators of psychological distress among U.S. and Canadian medical students. Acad Med 2006; 81(4): 354−73.
50. *Ozdemir H, Rezaki M.* General Health Questionnaire-12 for the detection of depression. Turk Psikiyatri Derg 2007; 18(1): 13−21. (Turkish)
51. *Al-Qaisy LM.* The relation of depression and anxiety in academic achievement among group of university students. Int J Psychol 2011; 3(5): 96−100.
52. *World Health Organization.* Health 2020: A European policy framework and strategy for the 21st century. Copenhagen (DK): World Health Organization; 2013.

Received on November 6, 2014.
Revised on January 4, 2015.
Accepted on January 12, 2015.
Online First December, 2015.

ORIGINAL ARTICLE

UDC: 615.322::[616-008.9-085+616-004.6-085
DOI: 10.2298/VSP140917148C

Effects of atorvastatin and artichoke leaf tincture on oxidative stress in hypercholesterolemic rats

Uticaj atorvastatina i tinkture lista artičoke na oksidativni stres kod pacova sa hiperholesterolemijom

Milkica Crevar-Sakač*, Zorica Vujić*, Jelena Kotur-Stevuljević†, Jasmina Ivanišević†, Zorana Jelić-Ivanović†, Marina Milenković‡, Milica Markelić§, Zoran Vujčić‖

***Department of Pharmaceutical Chemistry, †Department of Medical Biochemistry, ‡Department of Microbiology and Immunology, Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia; §Center for Electron Microscopy, Faculty of Biology, University of Belgrade, Belgrade, Serbia; ‖Department of Biochemistry, Faculty of Chemistry, University of Belgrade, Belgrade, Serbia**

Abstract

Backgroung/Aim. Since combining conventional drugs with herbal medicinal products is in current research focus and possible of great interest as therapy improvement way, the aim of this study was to determine the effects of well-established antiatherosclerotic drug atorvastatin (CAS number 134523-00-5) and commercially available artichoke leaf tincture (ALTINC), used as combined therapy, as well as to compare effects of these two treatments separately. **Methods.** Experimental animals were divided into five groups: the group I (the control group of rats fed with standard diet during 11 weeks), and the remaining 4 groups of rats (II, III, IV and V) fed with standard diet during the first week and then with hypercholesterolemic diet during the next 10 weeks. The group II of rats were left without treatment, while in the groups III, IV and V were rats treated *per os* with atorvastatin (1.15 mg/kg body wright − b.w.), ALTINC (0.1 mL/kg b.w.) and their combination in same doses, respectively, for the last six weeks. **Results.** The cholesterol rich diet led to pronounced hyperlipidemia which could not be overcame with the therapy. However, the therapy showed positive effects on abdominal aorta wall thickness and parameters of oxidative stress (malondialdehyde − MDA, proxidative-antioxidative balance − PAB) and antioxidative protection (reduced glutathione − GSH, paraoxanase 1 − PON1, superoxide dismutase − SODA SH groups), especially ALTINC was successful in oxidative status improvement. **Conclusion.** Separate treatments comparison showed that artichoke leaf tincture is very potent antioxidant with beneficial effects in early stages of atherosclerosis. Since atorvastatin and constituents of ALTINC probably have different mechanisms of action, simultaneous use of both therapies could be beneficial but should be further investigated since our results showed that ALTINC is less effective when used in combination with atorvastatin.

Key words:
cynara scolimus; atorvastatin calcium; atherosclerosis; oxidative stress; rats; treatment outcome.

Apstrakt

Uvod/Cilj. Savremena istraživanja sve više se okreću mogućnosti kombinovanja konvencionalne terapije sa biljnim lekovitim proizvodima. Cilj ovog istraživanja bio je praćenje efekata atorvastatina (CAS broj 134523-00-5), poznatog leka koji se koristi u terapiji hiperlipidemije, i tinkture lista artičoke primenjenih pojedinačno, kao i u obliku kombinovane terapije. **Metode.** Eksperimentalne životinje bile su podeljene u pet grupa: grupa I bila je kontrolna i činili su je pacovi hranjeni standardnom hranom za glodare tokom 11 nedelja, dok su preostale četiri grupe (II–V) činili pacovi koji su hranjeni standardnom hranom tokom prve nedelje, a potom u narednih 10 hranom bogatom holesterolom. Grupa II nije dobijala nikakav tretman, dok su III, IV i V posle 4. nedelje od početka uzimanja hrane bogate holesterolom, tokom narednih šest nedelja tretirane *per os*, redom: atorvastatinom u dozi od 1,15 mg/kg, tinkturom artičoke u dozi od 0,1 mL/kg i njihovom kombinacijom u istim dozama. **Rezultati.** Hrana bogata holesterolom izazvala je uznapredovalu

Correspondence to: Milkica Crevar Sakač, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Belgrade, Vojvode Stepe 450, 11 000 Belgrade, Serbia. Phone: +381 11 3951335. E-mail: frodo@pharmacy.bg.ac.rs

hiperholesterolemiju na koju terapija nije značajno delovala. Terapija je pokazala povoljan efekat na debljinu zida trbušne aorte, parametre oksidativnog stresa (malondialdehid − MDA, prooksidativni-antioksidativni balans − PAB) i antioksidativne zaštite (redukovani glutation − GSH, SH grupe, paraoksonazu1 − PON1, superoksid dismutazu − SOD). Tinktura lista artičoke pokazala se najboljom u poboljšanju oksidativnog statusa. **Zaključak.** Poređenje pojedinačne terapije pokazalo je da tinktura lista artičoke ima jako antioksidativno dejstvo i pokazuje povoljne efekte u početnim fazama aterosklerotskog procesa. S obzirom na to da atorvastatin i komponente tinkture lista artičoke verovatno deluju različitim mehanizmima, kombinovana primena može imati povoljne efekte, ali se mora dodatno ispitati jer dobijeni rezultati ukazuju da je tinktura lista artičoke manje efikasan kada se primenjuje zajedno sa atorvastatinom.

Ključne reči:
cynara scolimus; atorvastatin kalcijum; ateroskleroza; stres, oksidativni; pacovi; lečenje, ishod.

Introduction

Hyperlipidemia with plasma-elevated concentrations of cholesterol and low density cholesterol (LDL-C) is considered to be the cause of cardiovascular disease. Treatment of hyperlipidemia needs diet control, exercise and using lipid-lowering compounds such as drugs and diet.

The 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase (EC 1.1.1.88) inhibitors (statins) have emerged as the most important class of lipid-lowering agents. Through inhibition of HMG-CoA reductase, they restrict the rate-limiting step of cholesterol synthesis resulting in up-regulation of low density lipopotein (LDL) receptors on the cell membrane and reduction of atherogenic LDL consequences. Several clinical trials have demonstrated the beneficial effects of statins in cardiovascular disorders, extending beyond their effects on cholesterol level, in primary and secondary prevention settings [1, 2]. As a result, nowadays statins represent one of the most powerful agents for the treatment of cardiovascular events [3]. Statins also have many cholesterol-independent (pleiotropic) beneficial effects such as their antioxidative activity [1, 4, 5].

Although the side effects of statins are relatively low, they can cause rhabdomyolysis in rare cases [6]. Therefore, research to identify natural agents with lipid-lowering properties and with less or no adverse effects, especially medicinal plants, is warranted.

Artichoke (*Cynara scolymus* L.) is a plant which is widely grown in Mediterranean countries. Leaves of this plant are rich in antioxidant constituents. It contains many different polyphenolic compounds such as cynarin, chlorogenic acid, luteolin, apigenin [7, 8]. Different types of artichoke leaf extract were reported to have a cholesterol-reducing effect on hypercholesterolemic subjects [9, 10]. In addition, *in vitro* studies show that artichoke extract decreases the production of reactive oxygen species, oxidation of low-density lipoproteins and lipid peroxidation [11, 12].

It is not unusual that patients on statin therapy concomitantly take herbal medicinal product (e.g. extracts of artichoke leaf prodacts or another) known for its antioxidant properties, therefore it is important to investigate any possible synergistic or opposite action of these treatments. Comparing antioxidant properties of these two therapies can give answer the question if it is reasonable to suggest artichoke leaf product usage in the beginning stages of atherosclerosis.

Methods

Diets, chemicals and herbal product

In this experiment we used normal, commercial rat chow diet and special atherogenic diet prepared in our laboratory by adding cholesterol, sodium cholate and sunflower oil to commercial diet [13]. Cholesterol and sodium cholate hydrate were purchased from Sigma Aldrich (Dorset, United Kingdom). Sunflower oil was purchased from "Dijamant" (Zrenjanin, Serbia).

Concentrated phosphoric acid used for HPLC analysis was bought from J.T. Baker (Deventer, Netherland). Acetonitrile, HPLC grade, methanol, as well as cynarin and chlorogenic acid were purchased from Sigma Aldrich (Steinheim, Germany). Ultra pure water used for chromatography was prepared by means of TKA water purification system (Niederelbert, Germany).

Atorvastatin calcium standard used in the treatment was donated from Nobel Ilaç (Turkey).

Herbal product used in the experiment was commercial artichoke leaf tincture bought from the Institute for Medicinal Plant Research "Dr. Josif Pančić" (Belgrade, Serbia). Plant material used for tincture preparation was dried primary rosette of artichoke plant. The method of extraction carried out by manufacturer was single percolation during 24 hours. After that time extract was collected, left three days and then filtered. One part of plant material gives five parts of extract. Extraction was performed with mixture of ethanol and water (38:62, w/w).

All other chemicals used for biochemical assays during the experiment were of the highest purity available and were obtained from Sigma Aldrich (Dorset, United Kingdom).

Quantification of cynarin and chlorogenic acid in artichoke leaf tincture

Since the manufacturer had not provided information on the chemical composition of the tincture, the content of cynarin and chlorogenic acid was determined by HPLC method described in Ph.Eur. 7.0 monograph for Artichoke Leaf (Cynarae folium).

HPLC system used for experiment consisted of Agilent Technologies HP 1200 liquid chromatograph with binary pump and DAD detector (Agilent, Santa Clara, CA, SAD). A ZORBAX Eclipse Plus C18 Analytical (4.6 mm x 250 mm, 5 μm) (Agilent, Santa Clara, CA, SAD) column was used for

chromatographic separation of the compounds. The analytes were eluted by a gradient mobile phase system consisting of mobile phase A (phosphoric acid R : water R (0.5 : 99.5 v/v)) and mobile phase B (phosphoric acid R : acetonitrile R (0.5 : 99.5 v/v)). During the first minute after injection mobile phase was isocratic (92% mobile phase A and 8% mobile phase B). From the first to the twentieth minute linear change from 92% to 75% mobile phase A and from 8% to 25% mobile phase B was performed. For the next 13 minutes the ratio of mobile phases remained 75% mobile phase A and 25% mobile phase B. During the last two minutes of chromatografic run (33–35 minutes) linear change from 75% to 0% mobile phase A and from 25% to 100% mobile phase B was performed. The flow rate was 1.2 mL/min. The column temperature was 40ºC and detection was performed on 330 nm.

Reference solution of cynarin was prepared by dissolving 5 mg of standard substance in 50 mL of methanol and further transfering 2.5 mL of prepared solution to 10 mL volumetric flask and diluting to volume with methanol. Reference solution of chlorogenic acid was prepared in the same way. Concentrations of cynarin and chlorogenic acid in prepared standard solutions were 0.025 mg mL-1. A sample of the examined tincture for HPLC analysis was prepared by diluting 1 mL of tincture to 10 mL with the mixture of water and methanol (50:50, v/v). 1 mL of prepared solution was further diluted to 5 mL with the same diluent.

Animals

Male Wistar albino rats (7 weeks old, weighing 150–190 g at the beginning of the experiment) were used for the experiment. Animals were obtained from the Military Medical Academy Farm (Belgrade, Serbia). They were housed in groups of three in a controlled environment with 12 h light and dark cycles and were allowed free access to food and water.

All experimental procedures and protocols conformed to institutional guidelines for the care and use of animals in research No 5/10 (Ethics Committee of the University of Belgrade − Faculty of Pharmacy).

Study protocol

Rats were divided into five groups: the group I included control group rats fed with standard, normal diet (ND), and the remaining 4 groups (II, III, IV and V) were rats fed with normal pellet chow for one week, and then with hypercholesterolemic diet (standard pellet chow supplemented with 2% cholesterol, 3% sunflower oil and 1% sodium cholate) for the next ten weeks. The group II [atherogenic diet (AD) group] was left without treatment, while the groups III, IV and V consisted of atherogenic diet rats treated *per os* after four weeks without therapy, with atorvastatin (1.15 mg/kg b.w.), artichoke leaf tincture (0.1 mL/kg b.w.), and their combination, respectively, for the next six weeks. This therapy was applied to animals with a gastric probe. Atorvastatin was prepared as suspension in saline solution and artichoke leaf tincture was diluted with the same solvent for simplicity of application.

Doses of atorvastatin and ALTINC were chosen and calculated according to doses usually prescribed to humans and available data from experiments previously conducted by other researchers [14–16]. It was taken that grown up person of average body weight 70 kg takes 80 mg of atorvastatin daily, which means approximately 1.15 mg per kg body weight. In manual published by the manufacturer of commercial tincture is written that grown-up person should take 30 drops of tincture 3 times a day which means 90 drops in total daily. If average body weight of grown up person is 70 kg, that means 1.29 drops of ALTINC per kg body weight. For easier measurement and possible loss during treatment dose of ALTINC was 0.1 mL/kg b.w. These doses were extrapolated to experimental animals' average body weight. Body weights of rats were measured and doses of atorvastatin and ALTINC were recalculated once a week.

At the end of the experiment, animals from each group were fasted overnight, anesthetized by growing concentration of CO_2 and blood samples were collected directly from the heart in test tubes containing heparine and centrifuged 3,000 rpm for 15 min. Plasma samples were stored at -80ºC until they were analyzed. One part of the blood samples was hemolysed and erythrocyte hemolysates were kept at -80ºC until further analysis.

The liver of the rat was immediately removed, washed in ice-cold saline solution and blotted. Accurately-weighed pieces of tissue were minced and homogenized in nine volumes of 0.1 M phosphate buffer (pH 7.4), containing 1.15% KCl, using a polytron homogenizer. A portion of the homogenate was kept at -80ºC for the determination of malondialdehyde (MDA) and reduced glutathione (GSH) content.

Biochemical analysis

In plasma samples alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1), total proteins, glucose, total cholesterol (TC), triglycerides (TG) and high densitylipoprotein cholesterol (HDL-C) were assayed by routine enzymatic methods using an ILab 300+ analyzer (Instrumentation Laboratory, Milan, Italy) and Randox Laboratories (Armdore, UK) reagents. LDL-C was calculated using the Friedwald equation.

Oxidative stress parameters

Malondialdehyde (MDA) concentrations were measured using the thiobarbituric acid reactive substances (TBARS) assay employing the molar absorption coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ and spectrophotometry at 535 nm previously described by Girotti et al. [17]. Superoxidedismutase (SOD, EC 1.15.1.1) activity was measured according to the previously published method by Misra and Fridovich [18]. Reduced glutathione (GSH) content was measured according to the method of Jollow et al. [19]. Concentrations of sulfhydryl (SH) groups in plasma and erythrocytes were determined using 0.2 mmol/L 5.5′-dithiobis (2-nitrobenzoic acid) (DTNB) reported by Ellman [20]. Plasma pro-oxidant-antioxidant balance (PAB) was measured according to a previously published method [21]. Rates of paraoxonase

(PON1, EC 3.1.8.1) activity towards diazoxon were measured spectrophotometrically in plasma according to the method described by Richter and Furlong [22]. Diazoxon was purchased from Chem Service (West Chester, PA, USA). The activities are reported as IU/L.

Abdominal aorta wall thickness examination

For light microscopic examination, the lower portions of the abdominal aorta were dissected, formaldehyde-fixed and routinely embedded in paraffin. To distinguish between muscle cells and extracellular components of the aortic wall, 5 μm thick, the transversal sections of the aorta were rehydrated and stained according to the Azan trichrome staining method. Stained sections were analyzed microscopically (DMLB light microscope, Leica Microsystems, Mannheim, Germany). Wall thickness was measured using Image J software (NIH, Bethesda, Maryland, USA) by random selection of ten positions on the sections from every animal.

Statistical analysis

The characteristics of the study populations are presented as means ± standard deviations. Studied variables were compared using ANOVA with Tukey-Snedecor test as a *post hoc* analysis. Statistical analyses were performed using PASW® Statistic version 18 (Chicago, Illinois, USA) and Microsoft® Office Excel 2007. A value of p less than 0.05 was considered statistically significant.

Results

Content of cynarin and chlorogenic acid in artichoke leaf tincture

The determined content of both, cynarin and chlorogenic acid in investigated tincture was 0.2%.

Biochemical parameters

Table 1 indicates basic biochemical and lipid parameters, as well as animal body weight after six weeks of treatment. Results showed an increased concentration of liver enzymes (AST and ALT) in the groups on the atherogenic diet and atorvastatin (alone or in combination with ALTINC). The group that received only ALTINC showed the same level of liver enzyme activities as normally fed rats.

Lipid parameter concentrations were significantly higher in all the groups fed with atherogenic diet, except HDL-C concentrations which were similar in all the experimental groups and triglycerides which were lower in the groups on atherogenic diet. The group on atorvastatin treatment showed the lowest concentration, although with no statistical significance.

Abdominal aorta examination

The atherogenic diet led to a significant increase in abdominal aorta wall thickness comparing to rats fed with normal diet (Figure 1). It was found that all types of therapy had beneficial effect, but only the combination of atorvastatin and ALTINC decreased the thickness of the aortic wall with a statistically significant difference comparing to rats fed with atherogenic diet ($p < 0.001$) (Figure 1). Microscopic analysis of abdominal aorta (Figure 2A) revealed proper organization of muscular layers and elastic laminas maintained in most groups. Exceptions were the groups on AD and the group on AD + Ator (Figures 2B and C), where plaques were visible, distorting the proper organization of aortic wall tunica media.

Determination of oxidative stress and antioxidative parameters in liver tissue

To assess tissue oxidative status upon challenge with the atherogenic diet, as well as after antihyperlipidemic statin drug treatment, and under the influence of artichoke leaf

Table 1

Effect of atorvastatin (1.15 mg/kg body weight) and artichoke leaf tincture (ALTINC) (0.1 mL/kg body weight) treatment on general biochemical and lipid parameters after treatment

Parameter	ND	AD	AD + Ator	AD + ALTINC	AD + Ator + ALTINC	*p*
Weight gain (% comparing to weight at the beginning of the experiment)	154 ± 21	196 ± 31[a]	187 ± 18[a]	175 ± 27	191 ± 18[a]	≤ 0.05
ALT (IU)	39.5 ± 7.0	51.8 ± 12.8	56.2 ± 11.0	47.5 ± 12.2	64.3 ± 10.5	≤ 0.01
AST (IU)	122.8 ± 39.6	167.2 ± 19.1	205.4 ± 35.7[aa]	127.5 ± 52.8[c]	169.8 ± 19.3	≤ 0.01
tCHOL (mmol/L)	1.45 ± 0.19	2.96 ± 0.49	3.67 ± 1.75	4.48 ± 1.39[a]	3.80 ± 1.66	≤ 0.01
LDL (mmol/L)	0.64 ± 0.21	2.28 ± 0.53	3.06 ± 1.72	3.83 ± 1.45[a]	3.15 ± 1.67	≤ 0.01
HDL (mmol/L)	0.478 ± 0.057	0.446 ± 0.108	0.428 ± 0.064	0.442 ± 0.092	0.455 ± 0.075	0.86
nonHDL (mmol/L)	0.97 ± 0.14	2.51 ± 0.49	3.24 ± 1.74	3.49 ± 1.64	3.34 ± 1.68	≤ 0.05
TG (mmol/L)	0.737 ± 0.205	0.508 ± 0.114	0.382 ± 0.065	0.455 ± 0.126	0.422 ± 0.137	≤ 0.001
TP (g/L)	70.8 ± 7.8	71.5 ± 4.7	72.1 ± 1.4	69.2 ± 2.3	69.9 ± 1.6	0.780
GLU (mmol/L)	7.3 ± 1.5	7.1 ± 1.8	6.6 ± 0.8	7.8 ± 1.1	8.3 ± 2.0	0.362

ALT – alanine aminotransferase; AST – aspartate aminotransferase; tCHOL – total cholesterol concentration; LDL – concentration of cholesterol contained in low density lipoproteins; HDL – concentration of cholesterol contained in high density lipoproteins; nonHDL – concentration of cholesterol apart from cholesterol contained in high density lipoproteins; TG – concentration of triglycerides; TP – concentration of total proteins; GLU – concentration of glucose; ND – rats fed with normal diet; AD – rats fed with atherogenic diet, AD + Ator – rats fed with atherogenic diet treated with atorvastatin; AD + Ator + ALTINC – rats fed with atherogenic diet treated with atorvastatin and artichoke leaf tincture combination; AD + ALTINC – rats fed with atherogenic diet treated with artichoke leaf tincture.

Note: The results are given as mean ± standard deviation.

> *p* − values in the last column according to ANOVA; *p* − values according to *post-hoc* Tukey-Snedecor test: [a] $p < 0.05$, [aa] $p < 0.01$ *vs* ND; [c] $p < 0.05$ *vs* AD + Ator.

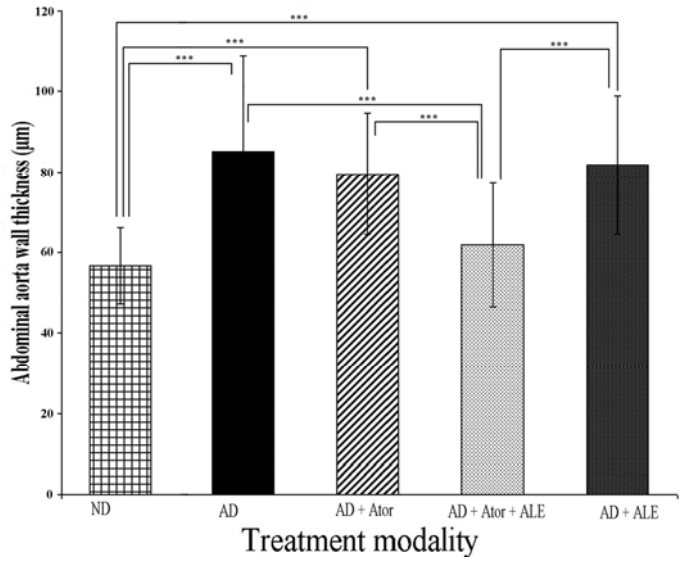


Fig. 1 − Average vaules of abdominal aorta wall thickness (µm) after six weeks of treatment.
ND – normal diet; AD – atherogenic diet; AD + Ator – atherogenic diet with atorvastatin treatment, AD + Ator + ALTINC – atherogenic diet with atorvastatin and artichoke leaf tincture (ALTINC) treatment; AD + ALTINC – atherogenic diet with artichoke leaf tincture treatment.
Each bar represents the mean ± SEM (n = 6). Statistical comparison was performed using ANOVA with Tukey-Snedecor test as *post hoc* analysis. The stars show a *p* value between groups connected with lines; ***$p < 0.001$.

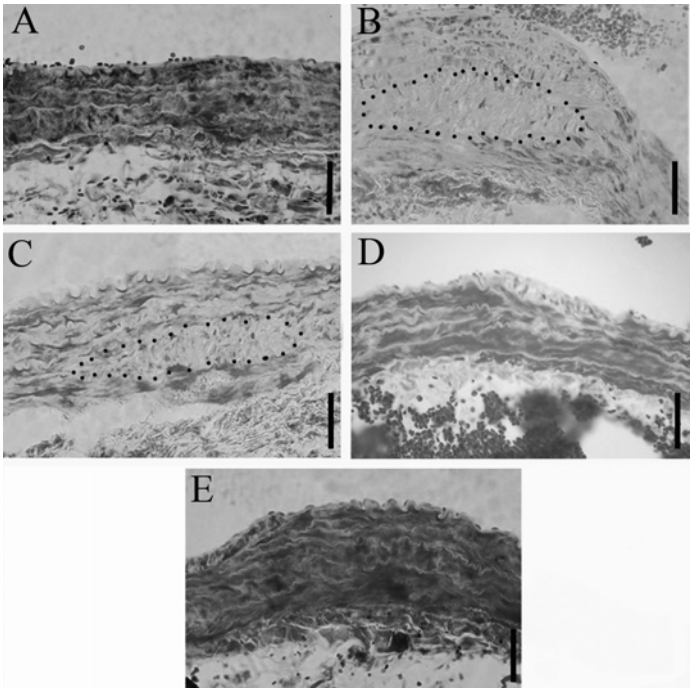


Fig. 2 − Representative photomicrographs of cross-sections through the abdominal aorta.
A – normal diet; B – atherogenic diet; C – atherogenic diet with atorvastatin treatment; D – atherogenic diet with atorvastatin and artichoke leaf tincture treatment; E – atherogenic diet with artichoke leaf tincture treatment.
The differences in *tunica media* thickness among the groups are observed. Also, in *tunica media* of rats on atherogenic diet and rats on atherogenic diet treated with atorvastatin, plaques are visible (spots marked area), leading to the distortion of proper organization of muscle layers and elastic laminas of *tunica media* (Azan trichrome staining, scale bars 50 µm).

tincture treatment we performed liver tissue oxidative stress analysis, i.e. MDA and GSH determination.

Malondialdehyde increased after the atherogenic diet. The therapy slightly lowered MDA, comparing with the group that was on the atherogenic diet, but without statistical significance (Figure 3). Our results showed a decrease in liver tissue GSH concentration after the atherogenic diet. Treatment with atorvastatin, ALTINC and the combination of both increased the concentration of GSH several fold (Figures 2D and 2E). Reduced glutathione was highest in the group that received ALTINC, far above GSH for animals on the ND ($p < 0.001$).

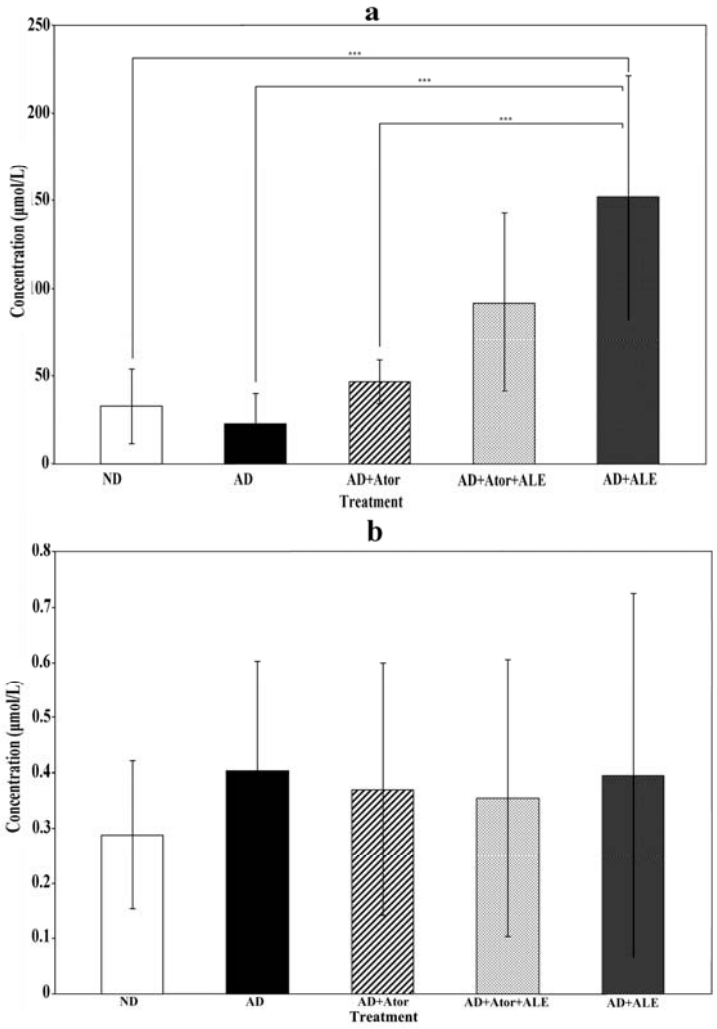


Fig. 3 − Oxidative stress status in liver tissue after six weeks of treatment.
a – glutathione (GSH) concentration; b – melondialdehyde (MDA) concentration; ND – normal diet; AD – atherogenic diet; AD + Ator – atherogenic diet with atorvastatin treatment; AD + Ator + ALTINC – atherogenic diet with atorvastatin and artichoke leaf tinctura treatment; AD + ALTINC – atherogenic diet with artichoke leaf tinctura treatment.
Each bar represents the mean ± SEM (n = 6). Statistical comparison was performed using ANOVA with Tukey-Snedecor test as *post hoc* analysis. The stars show a *p* value between groups connected with lines. ***$p < 0.001$.

Determination of oxidative stress and antioxidative parameters in plasma

Oxidative stress and antioxidative parameters were determined in plasma to estimate the oxidative stress changes in circulation (Table 2).

Malondialdehyde increased in all the treated groups ($p \leq 0.05$ compared with the ND group).

Prooxidative – antioxidative balance (PAB) value was higher in the groups on the AD and atorvastatin treatment regardless of whether it was administered alone or in combination with ALTINC ($p < 0.01$) compared with the ND group. The group that received only ALTINC showed values comparable with the group on the ND. Superoxide dismutase (SOD) activity was slightly lower in the group that was on the atherogenic diet. The therapy increased SOD activity (statistically significant effect of ALTINC, comparing with the groups on normal and atherogenic diets).

We found increased concentrations of total SH groups in all experimental groups compared with the ND group, although this increase was not statistically significant. The concentration of GSH significantly fell under the influence of AD. Atorvastatin and the combined treatment failed to increase GSH, but the ALTINC increased GSH significantly.

PON1 activity significantly decreased in the group given atherogenic diet. The therapy with either atorvastatin or ALTINC alone has no effect on the activity of paraoxonase. Only the combination of atorvastatin and ALTINC was able to maintain PON1 activity at baseline levels.

Determination of oxidative stress and antioxidative parameters in erythrocyte hemolysate

Oxidative stress and antioxidative parameters were measured in erythrocyte hemolysate to determine the oxidative-stress changes in blood cells (Table 3). There was no difference

Table 2

Effect of atorvastatin (1.15 mg/kg body weight) and artichoke leaf tincture (0.1 mL/kg body weight) treatment on parameters of oxidative stress and antioxidative protection in plasma after six weeks of the treatment

Parameters	ND	AD	AD + Ator	AD + ALE	AD + Ator + ALE	*p*
MDA (μmol/L)	0.472 ± 0.101	0.850 ± 0.762	0.610 ± 0.067[a]	0.962 ± 0.493[a]	1.223 ± 0.874[a]	0.172
PAB (HK units)	186 ± 57	368 ± 139[aa]	364 ± 115[aa]	266 ± 39[a, c]	367 ± 135[aa]	≤ 0.05
SOD (IU)	112 ± 4	109 ± 10	117 ± 5	119 ± 4[a, b]	115 ± 6	0.106
SH groups (mmol/L)	0.205 ± 0.085	0.248 ± 0.011	0.482 ± 0.316	0.553 ± 0.590	0.347 ± 0.023	0.215
GSH	32.7 ± 21.1	9.6 ± 2.7	10.2 ± 2.8[a]	33.7 ± 14.7[bb,cc]	20.0 ± 10.8[b, c]	≤ 0.01
PON1 (IU/L)	4785 ± 327	2785 ± 417[a]	1991 ± 473[aaa,b]	2441 ± 497[a]	3072 ± 662	≤ 0.01

MDA – concentration of malondialdehyde; PAB - prooxidative – antioxidative balance; SOD – activity of superoxide dismutase; GSH – concentration of reduced glutathione; PON1 – activity of paraoxonase1.

ND – rats fed with normal diet, AD – rats fed with atherogenic diet, AD + Ator – rats fed with atherogenic diet treated with atorvastatin, AD + Ator + ALTINC – rats fed with atherogenic diet treated with atorvastatin and artichoke leaf tincture combination, AD + ALTINC – rats fed with atherogenic diet treated with artichoke leaf tincture.

Note: The results are given as mean ± standard deviation.

***p* values in the last column according to ANOVA; *p* values according to *post-hoc* Tukey-Snedecor test: $^{a}p < 0.05$, $^{aa}p < 0.01$, $^{aaa}p < 0.001$ *vs.* ND; $^{b}p < 0.05$, $^{bb}p < 0.01$ *vs.* AD, $^{c}p < 0.05$, $^{cc}p < 0.01$ *vs.* AD + Ator.**

Table 3

Effect of atorvastatin (1.15 mg/kg body weight) and artichoke leaf tincture (0.1 mL/kg body weight) treatment on parameters of oxidative stress and antioxidative protection in erythrocyte hemolysate after six weeks of the treatment

Parameters	ND	AD	AD + Ator	AD + ALE	AD + Ator + ALE	*p*
MDA (μmol/L)	0.050 ± 0.006	0.030 ± 0.020	0.076 ± 0.031	0.188 ± 0.069	0.098 ± 0.056	≤ 0.01
SOD (IU/L)	3.08 ± 3.08	6.36 ± 4.05	6.57 ± 4.15	11.84 ± 3.44[a]	10.97 ± 1.64	≤ 0.01
SH groups (mmol/L)	0.171 ± 0.105	0.283 ± 0.162	0.254 ± 0.173	0.273 ± 0.126	0.324 ± 0.046	0.36
GSH (mmol/L)	0.91 ± 0.23	1.88 ± 0.83	2.54 ± 1.25	10.25 ± 10.39[aa,b,c]	4.24 ± 0.83	≤ 0.01

MDA – concentration od malondialdehyde; SOD – activity of superoxide dismutase; GSH – concentration of reduced glutathione; ND – rats fed with normal diet; AD – rats fed with atherogenic diet; AD + Ator – rats fed with atherogenic diet treated with atorvastatin; AD + Ator + ALE –with atherogenic diet treated with atorvastatin and artichoke leaf extract combination; AD + ALE – rats fed with atherogenic diet treated with artichoke leaf extract.

Note: The results are given as mean ± standard deviation.

***p* – values in last column according to ANOVA; *p* – values according to *post-hoc* Tukey-Snedecor test: $^{a}p < 0.05$, $^{aa}p < 0.01$ *vs* ND; $^{b}p < 0.05$ *vs* AD; $^{c}p < 0.05$ *vs* AD + Ator.**

in MDA levels and concentration of SH groups between all examined groups ($p > 0.05$).

SOD activity was significantly higher ($p < 0.05$) in the groups on the atherogenic diet regardless of the therapy. SOD activity was highest in the groups that had received ALTINC as treatment (alone or in combination with atorvastatin). All the groups receiving therapy showed higher GSH concentrations. The treatment with ALTINC led to the highest level of GSH ($p < 0.01$ comparing with the normal diet; $p < 0.05$ comparing with the atherogenic diet).

Discussion

Cholesterol feeding has often been used in experimental animals to study the pathogenesis of atherosclerosis. We supplemented a rat commercial diet with cholesterol, sodium cholate and sunlower oil as atherogenic compounds. Sodium cholate, which has an inhibitory action on hepatic *cholesterol 7-α hydroxylase* (EC 1.14.13.17) activity, is known to improve cholesterol absorption because of its emulsifying properties [23].

It is well-known that oxidative stress is a mechanism underlying pathogenesis of atherosclerosis. Our findings, which are in accordance with previous studies [23, 24], show that hypercholesterolemia disturbs PAB in favor of prooxidation in liver tissue (Figure 2).

Atorvastatin is the first choice drug for reducing lipids in hypercholesterolemic patients. Several studies have demonstrated that atorvastatin has no influence in high HDL-C concentration [5]. Atorvastatin has been found to exert beneficial cardiovascular effects independent of its ability to lower lipid amounts, possibly due to its antioxidant properties [1, 4, 13]. The mechanism involved in this phenomenon may be the ability of atorvastatin to reduce the production of reactive oxygen species [1]. Also, atorvastatin may play a role in protecting low density lipoprotein (LDL) and HDL from oxidation by increasing antioxidant activity of the HDL-associated enzyme PON1 [4, 5].

Different types of extracts of artichoke leaf have been considered to show antiatherogenic properties. It is reported that aqueous extract of artichoke leaf inhibits cholesterol biosynthesis in hepatocytes, decreases the oxidation of LDL, modulate endothelial functions and has choleretic activity [16]. Studies clearly show that cynarin is the main dicaffeoylquinic acid and chlorogenic acid is the major monocaffeoyilquinic acid, whereas luteolin-7-O-glucoside is the major flavonoid. Both caffeoylquinic acids and flavonoids present in artichoke extracts are considered to be responsible for its antiatherogenic actions. Llorach at al. [7] investigated different artichoke byproducts (raw artichoke, blanched artichoke and artichoke blanching waters). They extracted phenolic constituents (expressed as caffeic acid derivatives) with methanol and water. Both, methanol and water extracts from artichoke byproducts showed a high free radical scavenging activity as well as capacity to inhibit lipid peroxidation. Gebhardt and Fausel [2] performed in vitro tests and exposed cultured rat hepatocytes to oxidation and cytotoxicity with tert-butyl hydroperoxide in order to characterize the antioxidative and hepatoprotective properties of aqueous artichoke extracts. They added aqueous artichoke extracts to rat hepatocytes prior or simultaneously with tert-butylhydroperoxide. *In vitro* studies of Gebhardt and Fausel [12] have provided evidence that the antioxidant potential of aqueous extract of artichoke leaves is dependent on radical scavenging and metal ion chelating effects of its constituents such as cynarin, chlorogenic acid and flavonoids. However, pure constituents of artichoke leaf have been shown to produce less inhibitory activity on free radical production than any type of extract [11, 12].

In the present study the cholesterol rich diet led to an increase in the concentration of several lipid parameters (total cholesterol, LDL-C, nonHDL-C), though not all: triglycerides and HDL-C were unaffected. The therapy with atorvastatin and ALTINC did not reduce the concentration of lipid parameters, which was also noticed by other researchers [25] and which could be explained, at least in part, with supremacy of diet-induced lipids. Also, this therapy did not influence HDL-C which is consistent with the published data [4, 5].

Measurements of the abdominal aorta wall thickness have proven useful in predicting cardiovascular disease risk [26]. Increase in the thickness of the abdominal aorta wall in this study indicates the damage of the wall due to plaque formation in atherogenesis. Intake of AD led to the thickening of the aortic wall (Figure 1), as well as to plaque formation (Figure 2). Relatively short atorvastatin treatment failed to restore the aortic wall as shown in Figures 1 and 2. Treatment with ALTINC (alone and especially in combination with atorvastatin) managed to reduce abdominal aorta wall thickness and reversed plaque formed during atherogenesis. More favorable effect of ALTINC comparing to atorvastatin prove that ALTINC is better antioxidant. The effect of combination of atorvastatin and ALTINC shows that these two treatments have an additive effect.

Liver transaminases (ALT and AST) in the hypercholesterolemic rats were higher than in normal animals, which may be attributed to the injury of liver tissue [27]. We have noticed that treatment with atorvastatin in current work led to further increase in plasma transaminase levels. Previous research has shown that atorvastatin can induce a mild increase in ALT and AST activity. Long-term therapy in most cases does not lead to liver damage, although there are rare cases of acute hepatotoxicity induced by atorvastatin [27]. The therapy with ALTINC decreased plasma transaminase activity demonstrating its hepatoprotective effect, which is in accordance with previous studies [12].

Lipid peroxidation is a well-established mechanism of cellular injury and measurement of MDA is widely used as an indicator of oxidative stress in cells and tissues. Rats fed with atherogenic diet in this study showed increase in MDA concentration because of oxidative stress that occurred in atherogenesis [23]. This therapy did not reduce plasma MDA level as expected, presumably because the very concentrated cholesterol diet led to advanced lipid peroxidation, which probably could not be prevented by the antioxidant activity of atorvastatin and ALTINC during the relatively short time of the experiment. On the other side, all types of therapy led to a significant reduction in the concentration of MDA in liver tissue comparing to the group on atherogenic diet, indicating that atorvastatin and aqueous-ethanolic artichoke leaf

extract have antioxidant effects, as shown by previous researchers [1, 4, 12]. The combination of ALTINC and atorvastatin showed an even stronger effect in preventing lipoperoxidation (Table 2). MDA levels in erythrocyte hemolysate did not differ significantly among the groups, but positive effects of every type of therapy was noticed. The therapy with ALTINC had especially beneficial effects as in liver tissue.

Prooxidative-antioxidative balance was determined only in plasma and it increased upon atherogenic diet due to oxidative stress that occured. Atorvastatin therapy (alone or in combination with ALTINC) did not lead to a decrease of PAB as expected. Only therapy with ALTINC decreased PAB, which demonstrated antioxidative action of the plant extract (Table 3).

Our results show that any form of therapy increased the concentration of SH groups that play a role in neutralizing free radicals in plasma, as expected. The most effective treatment was with ALTINC. Changes in reduced glutathione levels were the same in all sample types (Tables 2 and 3). Atherogenic diet decreased the level of GSH, which was presumably used in neutralizing free radicals formed during the process of atherogenesis. The treatments in our experiment regenerated spent glutathione. ALTINC showed the strongest effect (Table 2) indicating strong antioxidant activity of some consituents, such as flavonoids and hydroxycinnamic acids to be effective hydrogen donors and metal chelators as shown by Zapolska-Downar et al. [28] who tested aqueous and ethanolic extracts of artichoke plant. Caffeic and chlorogenic acid have also been reported to stimulate hydroxyl radical formation and to have pro-oxidant activity on Cu^{2+}-induced LDL oxidation [28]. It has been demonstrated that aqueous and ethanolic extracts of artichoke plant protect LDL against oxidative modification *in vitro* [11]. Gebhardt and Fausel [12] have reported that aqueous extract of artichoke leaf inhibits the production of MDA induced by exposure of cultured hepatocytes to t-butylhydroperoxide (t-BHP). According to them, the observed antioxidative potential can be attributed to some well-known components of artichoke leaf extract, although their action cannot account for the full potency of the extract. In our study, antioxidant effect of ALTINC was obvious, especially when used alone in the treatment. Atorvastatin is extensively metabolized in the liver under the influence of the enzyme CP3A4 and therefore combinations in which it is used must be carefully selected since a lot of drugs is metabolized by the same enzymes [29]. Constituents of artichoke leaf tincture (cynarin and chlorogenic acid) are metabolized in the same manner. So, a possible interaction between ALTINC and atorvastatin could be on the metabolism level.

Paraoxonase has emerged as the component of HDL most likely to explain its ability to metabolize lipid peroxides and to prevent their accumulation in LDL. Paraoxonase immunoreactivity is known to be increasingly present in the arterial wall as atheroma advances [30]. Protection of LDL and HDL from oxidation is probably related to PON's ability to hydrolyze some oxidized phospholipids and/or cholesteryl linoleate hydroperoxides which are present in oxidized LDL. Other studies show that during LDL oxidation in the presence of PON1, the enzyme is partially inactivated [31]. This effect could be related to displacement of calcium ions (which are required for PON arylesterase/paraoxonase activities) by copper ion (in the copper ion oxidative system). In the free radical oxidation system, as well as in the copper ion system, free radicals formed during lipid peroxidation can also inactivate PON [32]. Our results in plasma samples confirm that PON1 is consumed during atherogenesis so its levels are lowest in the group on an atherogenic diet. Antioxidants in the treatment of atherosclerosis prevent oxidation of LDL cholesterol and thus preserve PON1 activity [32]. In our study, only ALTINC show this effect. Atorvastatin treatment has no significant effect on this enzyme activity which may indicate lower antioxidant potential of atorvastatin.

An increased antioxidant activity of SOD in erythrocytes was also found by Küçükgergin et al. [33] after treatment with artichoke dry extract dissolved in water which confirmed antioxidant properties of artichoke plant.

Conclusion

Treatment with artichoke leaf tincture and atorvastatin shows beneficial effects on oxidative stress parameters, but has little influence on basic lipid parameters. Investigated artichoke leaf tincture shows even better results in oxidative stress reduction than low-dose atorvastatin which is a widely-prescribed drug in the treatment of atherosclerosis. All the determined parameters show that artichoke leaf tincture (alone or in combination with atorvastatin) is very potent antioxidant in applied doses. Effects of specific constituents of ALTINC on oxidative damage should be further investigated. Since atorvastatin and constituents of ALTINC probably have different mechanisms of action, simultaneous use of both therapies could be beneficial but should be further investigated since our results show that ALTINC is less effective when used in combination with atorvastatin.

Acknowledgements

This work was financially supported by a grant from the Ministry of Education, Science and Technological Development, the Republic of Serbia (Project numbers OI 175035 and 172041).

R E F E R E N C E S

1. *Rosenson RS.* Statins in atherosclerosis: lipid-lowering agents with antioxidant capabilities. Atherosclerosis 2004; 173(1): 1−12.
2. *Wang C, Liu P, Liao JK.* Pleiotropic effects of statin therapy: molecular mechanisms and clinical results. Trends Mol Med 2008; 14(1): 37−44.
3. *Jasińska M, Owczarek J, Orszulak-Michalak D.* Statins: a new insight into their mechanisms of action and consequent pleiotropic effects. Pharmacol Rep 2007; 59(5): 483−99.
4. *Nagila A, Permpongpaiboon T, Tantrarongroj S, Porapakkham P, Chinwattana K, Deakin S,* et al. Effect of atorvastatin on paraoxonase1 (PON1) and oxidative status. Pharmacol Rep 2009; 61(5): 892−8.
5. *Paragh G, Törocsik D, Seres I, Harangi M, Illyés L, Balogh Z,* et al. Effect of short term treatment with simvastatin and atorvastatin on lipids and paraoxonase activity in patients with hyperlipoproteinaemia. Curr Med Res Opin 2004; 20(8): 1321−7.
6. *Maron DJ, Fazio S, Linton MF.* Current perspectives on statins. Circulation 2000; 101(2): 207−13.
7. *Llorach R, Espín JC, Tomás-Barberán FA, Ferreres F.* Artichoke (Cynara scolymus L.) byproducts as a potential source of health-promoting antioxidant phenolics. J Agric Food Chem 2002; 50(12): 3458−64.
8. *Wang M, Simon JE, Aviles IF, He K, Zheng Q, Tadmor Y.* Analysis of Antioxidative Phenolic Compounds in Artichoke (Cynara scolymus L.). J Agric Food Chem 2003; 51(3): 601−8.
9. *Ogier N, Amiot M, Georgé S, Maillot M, Mallmann C, Maraninchi M,* et al. LDL-cholesterol-lowering effect of a dietary supplement with plant extracts in subjects with moderate hypercholesterolemia. Eur J Nutr 2013; 52(2): 547−57.
10. *Qinna NA, Kamona BS, Alhussainy TM, Taha H, Badwan AA, Matalka KZ.* Effects of Prickly Pear Dried Leaves, Artichoke Leaves, Turmeric and Garlic Extracts, and Their Combinations on Preventing Dyslipidemia in Rats. ISRN Pharmacology 2012; 2012: 1−7.
11. *Brown JE, Rice-Evans CA.* Luteolin-rich artichoke extract protects low density lipoprotein from oxidation in vitro. Free Radic Res 1998; 29(3): 247−55.
12. *Gebhardt R, Fausel M.* Antioxidant and hepatoprotective effects of artichoke extracts and constituents in cultured rat hepatocytes. Toxicol In Vitro 1997; 11(5): 669−72.
13. *Vergnes L, Phan J, Strauss M, Tafuri S, Reue K.* Cholesterol and cholate components of an atherogenic diet induce distinct stages of hepatic inflammatory gene expression. J Biol Chem 2003; 278(44): 42774−84.
14. *Amin KA, Abd El-Twab TM.* Oxidative markers, nitric oxide and homocysteine alteration in hypercholesterolimic rats: role of atorvastatine and cinnamon. Int J Clin Exp Med 2009; 2(3): 254−65.
15. *Lunder M, Drevenšek G, Černe D, Marc J, Janić M, Šabovič M.* Treatment with low-dose atorvastatin, losartan, and their combination increases expression of vasoactive-related genes in rat aortas. J Cardiovasc Pharmacol Ther 2013; 18(2): 177−83.
16. *Rodriguez TS, Giménez DG, de la Puerta VR.* Choleretic activity and biliary elimination of lipids and bile acids induced by an artichoke leaf extract in rats. Phytomedicine 2002; 9(8): 687−93.
17. *Girotti MJ, Khan N, McLellan BA.* Early measurement of systemic lipid peroxidation products in the plasma of major blunt trauma patients. J Trauma 1991; 31(1): 32−5.
18. *Misra HP, Fridovich I.* The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972; 247(10): 3170−5.
19. *Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR.* Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic metabolite. Pharmacology 1974; 11(3): 151−69.
20. *Ellman GL.* Tissue sulfhydryl groups. Arch Biochem Biophys 1952; 82(1): 70−7.
21. *Alamdari DH, Ghayour-Mobarhan M, Tavallaie S, Parizadeh MR, Moohebati M, Ghafoori F,* et al. Prooxidant-antioxidant balance as a new risk factor in patients with angiographically defined coronary artery disease. Clin Biochem 2008; 41(6): 375−80.
22. *Richter RJ, Furlong CE.* Determination of paraoxonase (PON1) status requires more than genotyping. Pharmacogenetics 1999; 9(6): 745−53.
23. *Balkan J, Doğru-Abbasoğlu S, Aykaç-Toker G, Uysal M.* The effect of a high cholesterol diet on lipids and oxidative stress in plasma, liver and aorta of rabbits and rats. Nutr Res 2004; 24(3): 229−34.
24. *Kotur-Stevuljevic J, Memon L, Stefanovic A, Spasic S, Spasojevic-Kalimanovska V, Bogavac-Stanojevic N,* et al. Correlation of oxidative stress parameters and inflammatory markers in coronary artery disease patients. Clin Biochem 2007; 40(3−4): 181−7.
25. *Dalal I, Sengupta M, Paul S, Mishra A.* Comparative study of the effect of atorvastatin and garlic extract in experimentally induced hypercholesterolemia in rabbits. Int J Basic Clin Pharmacol 2013; 2(4): 397−402.
26. *Järvisalo MJ, Jartti L, Näntö-Salonen K, Irjala K, Rönnemaa T, Hartiala JJ,* et al. Increased aortic intima-media thickness: a marker of preclinical atherosclerosis in high-risk children. Circulation 2001; 104(24): 2943−7.
27. *Clarke AT, Mills PR.* Atorvastatin associated liver disease. Digest Liver Dis 2006; 38(10): 772−7.
28. *Zapolska-Downar D, Zapolski-Downar A, Naruszewicz M, Siennicka A, Krasnodębska B, Kołodziej B.* Protective properties of artichoke (Cynara scolymus) against oxidative stress induced in cultured endothelial cells and monocytes. Life Sci 2002; 71(24): 2897−908.
29. *Fujino H, Saito T, Tsunenari Y, Kojima J, Sakaeda T.* Metabolic properties of the acid and lactone forms of HMG-CoA reductase inhibitors. Xenobiotica 2004; 34(11−12): 961−71.
30. *Durak I, Ozbek H, Devrim E, Karagenç N, Ergüder IB.* Effects of cholesterol supplementation on antioxidant enzyme activities in rat hepatic tissues: possible implications of hepatic paraoxonase in atherogenesis. Nutr Metab Cardiovasc Dis 2004; 14(4): 211−4.
31. *Aviram M, Billecke S, Sorenson R, Bisgaier C, Newton R, Rosenblat M,* et al. Paraoxonase active site required for protection against LDL oxidation involves its free sulfhydryl group and is different from that required for its arylesterase/paraoxonase activities: selective action of human paraoxonase allozymes Q and R. Arterioscler Thromb Vasc Biol 1998; 18(10): 1617−24.
32. *Aviram M, Rosenblat M, Billecke S, Erogul J, Sorenson R, Bisgaier CL,* et al. Human serum paraoxonase (PON 1) is inactivated by oxidized low density lipoprotein and preserved by antioxidants. Free Radical Bio Med 1999; 26(7−8): 892−904.
33. *Küçükgergin C, Aydin A, Ozdemirler-Erata G, Mehmetçik G, Koçak-Toker N, Uysal M.* Effect of artichoke leaf extract on hepatic and cardiac oxidative stress in rats fed on high cholesterol diet. Biol Trace Elem Res 2010; 135(1−3): 264−74.

Received on September 17, 2014.
Revised on November 4, 2014.
Accepted on January 13, 2015.
Online First December, 2015.

SHORT COMMUNICATION

UDC: 355/359:616.89
DOI: 10.2298/VSP140114145J

Experience of the Air Medical Evacuation Team of Serbian Armed Forces in the United Nations Mission in the Democratic Republic of Congo – Deployment stress and psychological adaptation

Iskustvo pripadnika tima za vazdušnu medicinsku evakuaciju Vojske Srbije u misiji Ujedinjenih nacija u Demokratskoj Republici Kongo – stres i psihološka adaptacija u misiji

Danilo B. Joković*, Dragan Krstić*, Zvezdana Stojanović*, Željko Špirić*†

***Clinic for Psychiatry, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Background/Aim. Wars of the nineties in former Yugoslavia, Somalia, Rwanda imposed new tasks to the United Nations (UN) forces, such as providing humanitarian aid, protection of civilians, peacekeeping, and in many instances providing armed enforcement of peace. The aim of this study was an observational analysis of Serbian participation in the UNs Mission in the Democratic Republic of Congo with the emphasis on stress and coping techniques. **Methods.** Serbian contribution in this mission dates back to April 2003 till the present days with a military contingent consisting of six members as a part of Air Medical Evacuation Team. The observed stressogenous factors acted before arrival to the mission area and in the mission area. In this paper we analysed ways to overcome them. **Results.** The productive ways of overwhelming stress used in this mission were: honesty and openness in interpersonal communications, dedication to work, maintaining discipline and order, strict following of appropriate regime of work, diet, rest and recreation; regular communication with family and organizing and participation in various social, cultural and sports manifestations. **Conclusion.** This analysis indicates that out of all the observed factors, the most important is appropriate selection of personnel.

Key words:
stress, psychological; adaptation, psychological; medical missions, official; serbia.

Apstrakt

Uvod/Cilj. Sukobi 90-ih u bivšoj Jugoslaviji, Somaliji i Ruandi nametnuli su dodatne zadatke snagama Ujedinjenih nacija (UN) kao što su obezbeđivanje humanitarne pomoći, zaštita civila, očuvanje mira i primena oružanog očuvanja mira. Cilj ovog rada bio je sprovođenje opservacione analize srpskog učešća u misiji UN u Demokratskoj Republici Kongo sa akcentom na stres i tehnike za prevladavanje stresa. **Metode.** Srpsko učešće u ovoj misiji datira od aprila 2003. godine do danas, u vidu vojnog kontingenta Vojske Srbije od šest članova, koji čine tim za vazdušnu medicinsku evakuaciju. Stresogeni faktori delovali su pre dolaska u područje misije kao i u području misije. U ovom radu analizirani su načini za njihovo prevazilaženje. **Rezultati.** Produktivni načini prevladavanja stresa korišćeni u ovoj misiji bili su: iskrenost i otvorenost u međuljudskoj komunikaciji; posvećenost poslu; održavanje discipline i reda; striktno poštovanje odgovarajućih režima rada, ishrane, odmora i rekreacije; redovna komunikacija sa porodicom i organizovanje i učestvovanje u različitim društvenim, kulturnim i sportskim manifestacijama. **Zaključak.** Ova analiza pokazuje da je od svih razmatranih faktora najvažnija odgovarajuća selekcija ljudstva.

Ključne reči:
stres, psihički; adaptacija, psihološka; medicinske misije, zvanične; srbija.

Introduction

Wars of the nineties in former Yugoslavia, Somalia, Rwanda, have imposed new tasks to the United Nations (UN) forces, such as providing humanitarian aid, protection of civilians, peacekeeping, and in many instances providing armed enforcement of peace. These changes caused the increase in response to stress [1–3]. Factors that affect stress in peacekeeping operations are: individually conditioned factors, separation factors and factors of the mission [4, 5]. The de-

Correspondence to: Danilo Joković, Clinic for Psychiatry, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. Phone: 00381642295613. E-mail: ems_kinshasa_danilo@yahoo.com

gree of stress significantly correlated with mood, psychiatric symptoms and poor morale [6–10]. The most important dimensions of stress in soldier adaptation are: isolation, ambiguity, powerlessness, boredom, and threat [11–13]. Maladjustment can be viewed chronologically [14], from early up to late maladaptation reactions, and cyclically throughout the duration of deployment [15].

Air Medical Evacuation Team

Serbian participation in the United Nations (UN) Mission in the Democratic Republic of Congo (DRC) dates back to April 2003 up to the present days, with a military contingent consisting of six members – two doctors and four paramedics, as a part of Air Medical Evacuation Team (AMET), who are trained and equipped to set up two fully autonomous aeromedical evacuations. AMET has several tasks, the primary one being expert escort, monitoring vital signs, diagnosing and treatment of diseases during medical evacuation, casualty evacuation and medical repatriation by air and by land. The second task is provision of medical support during visits of official delegations and various military, political and cultural events. Also very important tasks are to secure logistical preconditions (repair of devices, procurement of new devices and procurement of expendables) for noninterrupted and continuous functioning and pleasant stay of a contingent in specific terms of the mission area, and, finally, regular, everyday communication and correspondence with the command in the mission and in country and performing numerous regular and *ad hoc* complex administrative duties.

The aim of this article was to illustrate deployment stress and psychological adaptation, as observed from the period of preparation up to the return home.

Methods

All data presented in this paper were obtained during the author's (DJ) involvement in the Serbian AMET mission as a member, where he participated twice, in 2005 and 2007. All the subjects were participants of AMET, six *per* term, totally 12. The shift took six months. AMET members were employed in the Serbian Armed Forces, and chosen for this mission. The data were collected through the author's observation of stressogenous factors, stress reactions and coping techniques in this mission, before arrival to the mission area (during a one-month preparation period), and during the whole stay in the mission area. The uniqueness of this article is that it talks about the first time in a 20-year history of Serbian participation in the UN Missions to deal with these issues. Table 1 shows some basic demographic data on the Serbian AMET.

Results and Discussion

Stressogenous factors

Stressogenous factors were numerous and can be divided into two groups: stressogenous factors before arrival to the mission area and stressogenous factors during the whole stay in the mission area (Table 2).

Stress reactions

Stress reactions in the mission were individually conditioned and therefore diverse. They include: emotions, somatic manifestations, interpersonal relationships, behavioral manifestations, as described by Logan [15] (Table 3).

Table 1

Demographic data of the Serbian Air Medical Evacuation Team (AMET)

Team data	Year	
	2005	2007
Number of members	6	6
Duration of rotation (months)	6	6
Military status of members		
officers	2	2
warrant officers	2	2
military employees	2	2
Number of previous deployments	1 (nurse)	1 (doctor and a nurse)
Gender of members		
male	4 (officers and warrant officers),	4 (officers and warrant officers),
female	2 (military employees)	2 (military employees)
Occupation of members		
general practitioners	2	2
nurses-paramedics	4	4
Average age of members (years)		
doctors	33	31.5
nurses	36.75	31
Marital status		
married	doctor and 2 nurses	3 nurses,
single	doctor and a nurse	2 doctors
divorced	nurse	nurse
Number of children		
2	doctor and 3 nurses	2 nurses
no children	doctor and a nurse	2 doctors and 2 nurses

Table 2

Stressogenous factors before arrival and during the stay in the mission area

Stressogenous factors before arrival to the mission area	Stressogenous factors during the stay in the mission area
Reactions of surroundings -misunderstanding, underestimation, sneering, envy, jealousy, maliciousness; Insufficient length and inadequate structure and focus of preparations-selection of candidates, prevailing of theory over practice, lack of focusing in training for specific tasks in the mission, lack of information on conditions in the Democratic Republic of Congo (DRC), incomplete knowledge of rights and obligations, long period between completion of training and departure followed by uncertainty about term; Unresolved issues of advance payments and reimbursements, untimely and incomplete supply with uniforms, equipment, protective equipment, medicines, vaccines, performing regular duties at workplace up to the point of departure	Mutual relationships; Culture shock; Accommodation; Climate; Health threats; Security risks; Difficulties in communication with family and superior command; Delays in payment of fees in Serbia and in the DRC; Imprecisely defined obligations and rights in the mission; Ride in terms of continuous congestion of roads in a disastrous situation; Medical evacuation by air with the use of aircraft not dedicated and equipped for this purpose and whose functioning, accuracy and timeliness of maintenance were often questioned; Contact with patients during medical evacuations on whose medical condition, most of the time there was not enough information and who were diagnosed and treated for the first time.

Table 3

Stress reactions in the mission

Emotions	Somatic manifestations	Interpersonal relationships	Behavioral manifesations
Feelings of sadness, rage, excitement, restlessness, anxiety, tension, frustration, embitterness, resentment, anger, numbness, confusion, disorganization, indecisiveness, loneliness, vulnerability, irritability, senselessness, despair, hopelessness, impatience, omnipotence, unlimited freedom.	Fluctuations in energy level and mood; Disturbances of sleep and appetite; Polymorphic somatic complaints; Fear of diseases manifested through multiple repetition of tests for infectious diseases (e.g. Malaria test).	Disturbed interpersonal relations in range from gossip, tricks, verbal disputes, creating clans, plotting and physical conflicts; Violations of rules and regulations of Serbian army and the UN-refusal to wear uniform, growing beard, impropriate behavior, conflicts with other UN personnel, abuse of UN property, refusal to follow orders, theft.	Excessive use of alcohol; Excessive use of nicotine; Occasional use of illicit drugs; Lack of social inhibitions; Promiscuous behaviour; Use of sex workers' services.

UN – United Nations.

Coping techniques

Productive ways of coping with stress, by the author's personal experience, were: honesty and openness in interpersonal communication, respect and fairness in the superior-subordinate relationship, smoothing misunderstandings, dedication to working for achievement of personal and mutual progress and well-being, professional execution of duties, maintaining discipline and order, strict adherence to the corresponding regime of work, nutrition, rest and recreation, regular communication with family, organizing and participating in various social, cultural and sports activities. His experience was in accordance with data from the literature and confirms that "only members with a high level of mental health will make success in peacekeeping operations in Africa" [16].

Recommendations

Although this engagement was specific, there were no substantial differences in deployment stress and psychological adaptation already described in the literature concerning the participation of medical personnel, in general. Factors that influenced deployment stress reactions and coping tech-

niques were very similar for both teams. The author was the member of both teams, as well as the nurse. The author's experience is that the best way around this mission showed younger, unmarried, children less, male doctors, officers, and middle-aged, married, with children, female, paramedics, military employees. The recommendations for stress prevention in this mission besides crucial factors are selection of personnel, also adequate training, equipping with necessary devices, then improvement of communication capabilities with command and families and improvement of living and working conditions, and, finally, maintaining exemplary discipline with correct interpersonal relationships and designing constructive leisure activities.

Conclusion

Appropriate selection of mentally stable, mature personality, intelligent, open minded, without prejudice, communicative, resourceful, wise, brave, courageous, devoted and motivated, persistent, competent, skilled and well-trained persons is a guarantee for successful adaptation to the Serbian AMET mission.

R E F E R E N C E S

1. *Hawthorne G, Sim M, McKenzie D.* Post-peacekeeping: The long-term health status of veteran peacekeepers. Melbourne: Australian Centre for Posttraumatic Mental Health; 2005.
2. *Bartone P, Adler A.* A model for soldier psychological adaptation in peacekeeping operations. Proceedings of the 36th Annual conference of the International Military Testing Association; Rotterdam, the Netherlands; 1994 October 25−7; Heidelberg, Germany: U.S. Army Medical Research Unit – Europe, Walter Reed Army Institute of Research; 1994
3. *Nasveld P, Cotea C, Pullman S, Pietrzak E.* Effects of deployment on mental health in modern military forces: A review of longitudinal studies. J Military Veteran Health 2013; 21(1): 14−23.
4. *Bartone P, Adler A.* Peacekeeping operations: Psychological Preparation. Heidelberg, Germany: US: Army Medical Research Unit − Europe, Walter Reed Army Institute of Research; 1995.
5. *Orsillo S, Roemer L, Litz B, Ehlich P, Friedman M.* Psychiatric symptomatology associated with contemporary peacekeeping: an examination of post-mission functioning among peacekeepers in Somalia. J Trauma Stress 1998; 11(4): 611−25.
6. *Kavanagh J.* Stress and performance: a review of the literature and its applicability to the military. Santa Monica: RAND Corporation; 2005.
7. *Sareen J, Cox BJ, Afifi TO, Stein MB, Belik S, Meadows G,* et al. Combat and peacekeeping operations in relation to prevalence of mental disorders and perceived need for mental health care: findings from a large representative sample of military personnel. Arch Gen Psychiatry 2007; 64(7): 843−52.
8. *Garrido M, Muñoz M.* Morale as a protection factor against Mission related stress Human dimensions in military operations - Military leaders' strategies for addressing stress and psychological support. Proceedings of the Symposium; 2006 April 24-26; Brussels, Belgium; Paris: NATO Science and Technology Organization; 2006.
9. *Wong A, Escobar M, Lesage A, Loyer M, Vanier C, Sakinofsky I.* Are UN peacekeepers at risk for suicide. Suicide Life Threat Behav 2001; 31(1): 103−12.
10. *Bartone PT.* Resilience under military operational stress: can leaders influence hardiness. Mil Psychol 2006; 18(Suppl): 131−48.
11. *Bartone P, Adler A, Vaitkus M.* Dimensions of psychological stress in peacekeeping operations. Mil Med 1998; 163(9): 587−93.
12. *Johansson E, Larsson G.* A model for understanding stress and daily experiences among soldiers in peacekeeping operations. International Peacekeeping 1998; 5(3): 124−41.
13. *Shigemura J, Nomura S.* Mental health issues of peacekeeping workers. Psychiatry Clin Neurosci 2002; 56(5): 483−91.
14. *Hall D.* Stress, suicide, and military service during Operation Uphold Democracy. Mil Med 1996; 161(3): 159−62.
15. *Logan VK.* The Emotional cycle of deployment. Camp Hill, PA: Center For Safe Schools; 1987.
16. *Van Dyk G.* Strategic challenges for African Armed Forces for the next decade. Stellenbosch: Sun Press; 2008.

Received on January 14, 2014.
Revised on January 25, 2015.
Accepted on January 27, 2015.
Online First December, 2015.

SHORT COMMUNICATION

UDC: 616.1-089:616.126.32-08
DOI: 10.2298/VSP141117024N

Percutaneous implantation of self-expandable aortic valve in high risk patients with severe aortic stenosis: The first experiences in Serbia

Perkutana implantacija samooslobađajuće aortne valvule kod visokorizičnih bolesnika sa teškom aortnom stenozom: prva iskustva u Srbiji

Milan A. Nedeljković*†, Branko Beleslin*†, Milorad Tešić*†, Vladan Vukčević*†, Goran Stanković*†, Siniša Stojković*†, Dejan Orlić*†, Ilija Bilbija‡, Miloš Matković‡, Tijana Simić§, Nemanja Menković‖, Igor Mrdović*†, Gian Paolo Ussia¶, Zoran Perišić, Momčilo Babić††**

***Clinic for Cardiology, ‡Clinic for Cardiac Surgery, §Center for Anesthesiology and Reanimation, ‖Clinic for Radiology, Clinical Center of Serbia, Belgrade, Serbia; †Faculty of Medicine, University of Belgrade, Belgrade, Serbia; ¶Department of Cardiovascular Disease, Tor Vergata University of Rome, Rome, Italy; **Clinic for Cardiovascular Diseases, Clinical Center Niš, Faculty of Medicine, University of Niš, Niš, Serbia; ††National Health Insurance Fund, Belgrade, Serbia;**

Abstract

Background/Aim. Aortic stenosis (AS) is the most common valvular heart disease in elderly people, with rather poor prognosis in symptomatic patients. Surgical valve replacement is the therapy of choice, but a significant number of patients cannot undergo surgical procedure. We presented initial experience of transcatheter aortic valve implantation (TAVI) performed in Catheterization Laboratory of the Clinic for Cardiology, Clinical Center of Serbia. **Methods.** The procedures were performed in 5 patients (mean age 76 ± 6 years, 2 males, 3 female) with severe and symptomatic AS with contraindication to surgery or high surgical risk. The decision to perform TAVI was made by the heart team. Pre-procedure screening included detailed clinical and echocardiographic evaluation, coronary angiography and computed tomography scan. In all the patients we implanted a self-expandable aortic valve (Core Valve, Medtronic, USA). Six months follow-up was available for all the patients. **Results.** All interventions were successfully performed without significant periprocedural complications. Immediate hemodynamic improvement was obtained in all the patients (peak gradient 94.2 ± 27.6 to 17.6 ± 5.2 mmHg, $p < 0.001$, mean pressure gradient 52.8 ± 14.5 to 8.0 ± 2.1 mmHg, $p < 0.001$). None of the patients developed heart block, stroke, vascular complication or significant aortic regurgitation. After 6 months, the survival was 100% with New York Heart Association (NYHA) functional improvement in all the patients. **Conclusion.** This successful initial experience provides a solid basis to treat larger number of patients with symptomatic AS and high surgical risk who are left untreated.

Key words:
aortic valve stenosis; transcatheter aortic valve replacement; severity of illness index; risk factors; cardiac surgical procedures.

Apstrakt

Uvod/Cilj. Aortna stenoza (AS) je najčešća mana zaliska kod odraslih, sa prilično lošom prognozom kod simptomatskih bolesnika. Hirurška zamena aortnog zaliska je terapija izbora, ali kod značajnog broja bolesnika ona nije izvodljiva. Prikazali smo inicijalno iskustvo sa transkateterskom implantacijom aortnog zaliska *transcatheter aortic valve replacement* (TAVR) koja je izvedena u sali za kateterizaciju na Klinici za kardiologiju Kliničkog centra Srbije. **Metode.** Procedura je urađena kod 5 bolesnika (srednje godine starosti 76 ± 6 godina, 2 muškarca i 3 žene) kod bolesnika sa teškom i simptomatskom AS koji su imali ili kontraindikacije za hiruršku zamenu aortnog zaliska ili visok rizik od hirurške procedure. Sve odluke o primeni TAVR doneo je kardiohirurški tim. Pregled bolesnika pre procedure obuhvatao je detaljni klinički i ehokardiografski pregled, koronarnu angiografiju i kompjuterizovanu tomografiju srca i vaskularnog sistema. Kod svih bolesnika implantiran je samooslobađajući aortni zalistak (CoreValve, Medtronic, USA). Šestomesečno praćenje je sprovedeno kod svih bolesnika. **Rezultati.** Sve intervencije uspešno su izvedene, bez značajnih periproceduralnih komplikacija. Neposredno hemodinamsko poboljšanje postignuto je kod svih bolesnika (maksimalni gradijent priti-

Correspondence to: Branko Beleslin, Clinic for Cardiology, Clinical Center of Serbia, Višegradska 26, 11 000 Belgrade, Serbia. Phone: +381 63 8328 690. E-mail: branko.beleslin@gmail.com

ska: 94.2 ± 27.6 *vs* 17.6 ± 5.2 mmHg, $p < 0.001$; srednji gradijent pritiska: 52.8 ± 14.5 *vs* 8.0 ± 2.1 mmHg, $p < 0.001$). Nijedan bolesnik nije imao srčane blokove, moždani udar, vaskularne komplikacije ili značajnu aortnu regurgitaciju posle intervencije. Posle 6 meseci, preživljavanje je bilo 100%, a funkcionalno poboljšanje je postignuto kod svih bolesnika. **Zaključak.** Ovaj inicijalni uspeh predstavlja dobru osnovu za lečenje većeg broja bolesnika sa simptomatskom AS koji imaju visok hirurški rizik, a do sada nisu bili adekvatno lečeni.

Ključne reči:
zalistak, aortni, stenoza; zalistak aorte, transkateterska zamena; bolest, indeks težine; faktori rizika; hirurgija, kardijalna, procedure.

Introduction

Aortic valve stenosis (AS) is becoming a common disease in elderly population. In patients with symptomatic severe AS there is unfavorable prognosis with survival rates of only 15–50% in 5 years [1]. Surgical valve replacement is the therapy of choice in patients with symptomatic AS, but the mortality after isolated surgical procedures is 1–3% in patients under 70 years, and 4–8% above 70 years [1]. In clinical practice at least 30% of patients with severe symptomatic AS do not undergo surgery for replacement of the aortic valve, due to advanced age, frequent comorbidities and frailty [1, 2]. An alternative and less invasive therapy – transcatheter aortic valve implantation (TAVI) was proposed and initiated in 2002 [3], and achieved in short period clinical acceptance, with more than 150,000 procedures done worldwide [4]. The initial experience demonstrated that TAVI has significant impact on prolongation and quality of life in inoperable and high risk patients with symptomatic AS, and as such, recognized by ESC guidelines for valve disease and recommended in high-risk surgical patients [1–3]. Currently, we have the 2 valve modalities including self-expandable and balloon-expandable valve with similar performance and success rate [2, 5–7]. The first self-expanding CoreValve (Medtronic, USA) has been registered in Serbia and here we presented our initial experience of TAVI in first 5 patients performed in Catheterization Laboratory of the Clinic for Cardiology, Clinical Center of Serbia.

Methods

Study population

Implantation of CoreValve was performed between April and May 2014 in the first 5 patients (mean age 76 ± 6 years) with severe symptomatic AS, after evaluation by the heart team consisting of invasive cardiologist, cardiac surgeon and non-invasive cardiologist.

According to the current European guidelines for valvular heart disease [1], TAVI is recommended in patients with symptomatic severe AS with contraindication to surgical procedure or high surgical risk [Society of Thoracic Surgeons (STS) score ≥ 10% and Logistic EuroSCORE ≥ 20%], without short life expectancy due to comorbidities or frailty. The decision for TAVI should always be provided by the heart team [8], and currently performed in hospitals with surgical facilities. Exclusion criteria for TAVI included clinical (short life expectancy, significant comorbidities, as well as severe coronary and other valvular heart disease that can be treated only by surgery) and anatomical (inappropriate size of the annulus and ascendent aorta, thrombi in the left ventricle, active endocarditis, high risk of coronary artery obstruction, complex thrombotic plaques in the aorta, and unavailable adequate vascular access) [1].

All the patients were informed about the risks and benefits of the procedure and provided informed consent for TAVI. All the procedures were performed with the guidance of the experienced proctor for TAVI (GP.U).

Study procedures and the device

Preprocedure screening included detailed clinical examination, echocardiographic overview of the function and dimensions of the heart, function of the valves, dimensions of the aorta with evaluation of severity of aortic stenosis (Figure 1), coronary angiography with percutaneous coronary intervention (PCI) at least 1 month prior to TAVI, computed tomography (CT) scan for precise measurements of diameters of the left ventricular outflow tract, aortic annulus (Figure 2A), sinus Valsalva (Figure 2B), ascending aorta (Figure 2C), the distance from the annulus to the orifice of coronary arteries (Figure 2D), in addition to sinus Valsalva height. Also, vascular access including the dimensions and appearance of femoral and subclavian arteries was done with CT scan, as well as full anesthesiological work-up of the patient prior to the procedure.

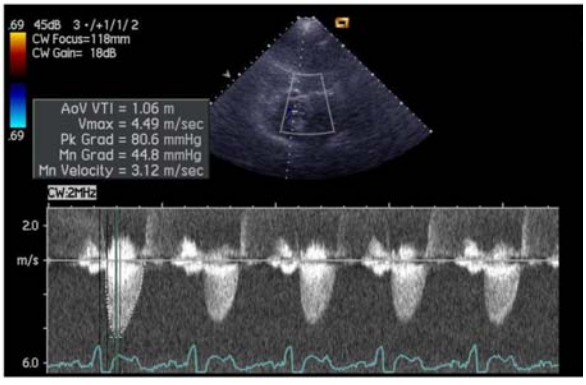


Fig. 1 – Transthoracic echocardiography – gradient across the aortic valve before transcatheter aortic valve implantation.

The CoreValve three leaflet bioprosthesis is made from porcine pericardium attached to nitinol frame (Figure 3). Nitinol frame allows crimping of the valve into the 18F profile loading system, which self expands into the full predesigned

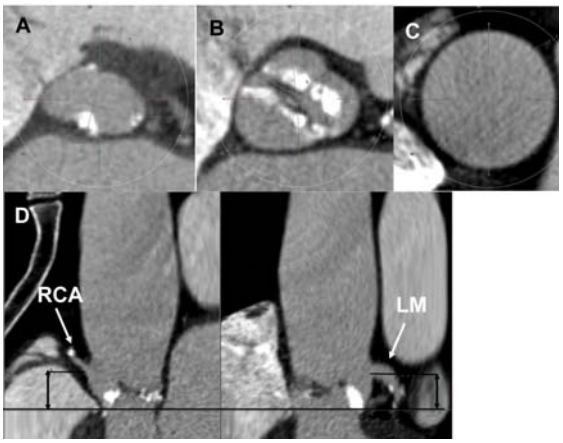


Fig. 2 – Computed tomography examination: A) Diameters of the aortic annulus; B) Diameters of the sinus Valsalva; C) Diameters of the ascending aorta; D) Distance from the annulus to orifice of the left main (LM) and right coronary artery (RCA).

Fig. 3 – The CoreValve three leaflet bioprosthesis – porcine pericardium attached to nitinol frame. Courtesy of Medtronic.

form and after implantation extends from the left ventricular outflow tract across the aortic annulus into the aortic root. The valve is available in three dimensions of 26, 29 and 31 mm diameters, and valve size selection is based on the precise measurement of the aortic annulus and root by echocardiography and most importantly by CT. The procedure is performed in catheterization laboratory under deep analgosedation or general anesthesia, either with pure percutaneous femoral approach or surgical incision of femoral artery. In cases in which femoral artery is too small or too tortuous, the procedure can be performed through subclavian artery. From the other inguinal groin femoral artery and vein, pigtail catheter is advanced into ascending aorta, whereas temporary pacemaker is placed into the right ventricle. After insertion of 18F sheet into right femoral or subclavian artery, left Amplatz catheter was advanced to aortic root to pass the aortic orifice first with soft wire and then exchanged for the Super Stiff Amplatz wire. Prior to implantation, a calcified valve is predilated with a large balloon under rapid ventricular pacing from right ventricle (Figure 4A). Aortic valve is then advanced and deployed under angiographic guidance from the pigtail catheter positioned in the aortic root (Figure 4B). After successful aortic prosthesis implantation, aortography was performed for evaluation of aortic regurgitation along with assessment of left ventricular pressures and curves (Figure 4C). All cases were finished by femoral suture performed by the surgeons, and transthoracic echocardiographic evaluation of position and function of aortic valve, residual aortic regurgitation and right ventricle pressures (Figure 5). Immediately after the procedure, all the patients were transferred to cardiac care unit for close clinical and hemodynamic monitoring for the next 2 to 3 days. The premedication included aspirin and clopidogrel for few days prior to the intervention, with continuation for the next 3–6 months.

Statistical analysis

Continuous variables were presented as mean ± standard deviation. Categorical variables were presented as frequencies in percentages. Statistical significance was calculated by Students *t*-test, or χ^2 for categorical variables. $p < 0.05$ was considered statistically significant.

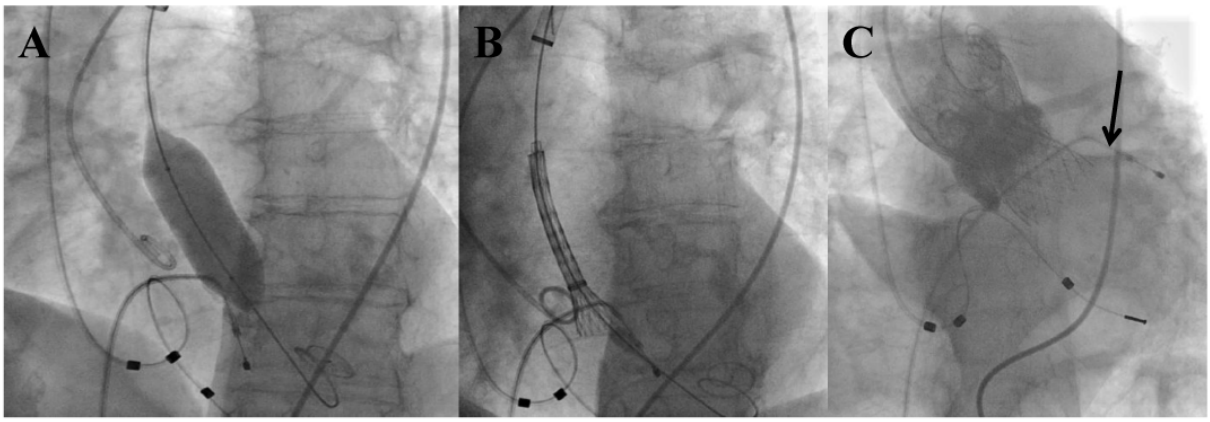


Fig. 4 – A) Predilatation of the native aortic valve with a balloon; B) Deployment of the CoreValve under angiographic guidance from the pigtail catheter positioned in the aortic root; C) Final aortography, with mild paravalvular aortic regurgitation (arrow).

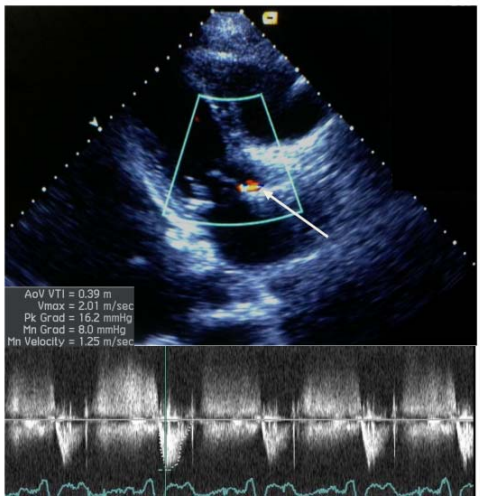


Fig. 5 – Transthoracic echocardiography – gradient across the CoreValve and trivial paravalvular regurgitation (arrow) after the transcatheter aortic valve implantation procedure.

Results

Baseline clinical and echocardiographic characteristics of the patients are presented in Table 1. All the patients were symptomatic prior to intervention with 80% of the patients in New York Heart Association (NYHA) class II-III. Common comorbidities were present in all the patients including mostly moderate to severe impairment of renal function, as well as previous cerebrovascular events, diabetes, previous thoracotomy and porcelain aorta in individual patients. High surgical risk that precluded cardiac surgery was estimated as a STS score of 16.3 ± 1.4 and LogEuroScore of 21.98 ± 9.87. All the patients had preserved left ventricular dimensions and function with the mean aortic valve area of 0.71 ± 0.12 cm^2.

Three of the patients had coronary artery disease, successfully treated previously with stenting. Control angiography demonstrated patent coronary arteries without significant new lesions or restenosis in all the three patients. There were no significant coronary artery stenosis in the other two patients.

In three patients procedure was performed through femoral artery, and in two patients left subclavian artery was used as entry site. Immediate hemodynamic improvement was achieved in all the patients, with decrease of peak pressure gradient from 94.2 ± 27.6 mmHg to 17.6 ± 5.2 ($p = 0.003$), and mean pressure gradient from 52.8 ± 14.5 mmHg to 8.0 ± 2.1 mmHg ($p = 0.002$). As indirectly measured from transthoracic echocardiography immediately after the procedure inside catheterization laboratory, systolic right ventricle pressure also decreased from 63 ± 15 to 40 ± 7.3 mmHg ($p = 0.020$) (Table 2). After the procedure all the patients had only mild aortic regurgitation. None of the patients suffered from any severe vascular complications, stroke,

Table 1

Clinical and echocardiographic characteristics of the patients (n = 5)

Variable	Value
Age (years), $\bar{x}$ ± SD	76 ± 6
Male/female, n (%)	2/3 (40/60)
Atrial fibrillation, n (%)	1 (20)
Left bundle branch block, n (%)	0
Chronic obstructive pulmonary disease, n (%)	0
Previous thoracotomy, n (%)	1 (20)
Chromic renal failure, n (%)	
none	1 (20)
moderate	3 (60)
severe	1 (20)
Previous cerebrovascular event, n (%)	1 (20)
Hypertension, n (%)	5 (100)
Diabetes, n (%)	2 (40)
Porcelain aorta, n (%)	1 (20)
Peripheral vascular disease, n (%)	2 (40)
Previous coronary artery disease, n (%)	3 (60)
Syncope, n (%)	1 (20)
Previous pacemaker, n (%)	2 (40)
STS score, $\bar{x}$ ± SD	16.3 ± 1.4
LogEuroScore, $\bar{x}$ ± SD	21.98 ± 9.87
Aortic area (cm^2), $\bar{x}$ ± SD	0.71 ± 0.12
LV end-diastolic dimension (mm), $\bar{x}$ ± SD	54 ± 5
LV systolic dimension (mm), $\bar{x}$ ± SD	33 ± 7
Ejection fraction (%), $\bar{x}$ ± SD	65 ± 5

STS – Society of Thoracic Surgeon; LV – left ventricle; $\bar{x}$ – mean value; SD – standard deviation.

Table 2

Clinical and echocardiographic characteristics of the patients – before the intervention and 30 days follow-up

Variable	Before intervention	After	*p*
NYHA Class, n (%)			
I-II	1 (20)	4 (80)	0.058
III-IV	4 (80)	1 (20)	
Peak pressure gradient (mmHg), $\bar{x}$ ± SD	94.2 ± 27.6	17.6 ± 5.2	0.003
Mean pressure gradient (mmHg), $\bar{x}$ ± SD	52.8 ± 14.5	8.0 ± 2.1	0.002
Systolic right ventricle pressure (mmHg), $\bar{x}$ ± SD	63 ± 15	40 ± 7.3	0.020

NYHA – New York Heart Association; $\bar{x}$ – mean value; SD – standard deviation.

transient ischemic attacks, heart block or other procedure complications, except for the one patient who developed in-hospital gastrointestinal bleeding due to erosive gastroduodenitis which was successfully treated with blood transfusion and proton pump inhibitors. The mean hospital stay was 14 ± 10 days. None of the patient died within 30 days and 6 months follow-up, and symptoms and quality of life improved in all the patients.

Discussion

Initial results with percutaneous implantation of self-expandable aortic valve in patients with symptomatic aortic stenosis with high surgical risk are promising and confirm previous excellent experience and data on TAVI [9, 10]. The technique appeared to be safe, feasible, effective and successful in reducing large pressure gradient and symptoms in patients with severe AS [11, 12]. Our initial results on survival are excellent, with complication of gastrointestinal bleeding occurring only in one patient. However, this complication was associated with concomitant antithrombotic and anticoagulant therapy and not to procedure *per se*. Of course, the initial results need to be extended with consistent application and performance as this highly sophisticated procedure requires considerable experience and learning curve. The other point is that the prevalence of degenerative AS is linked to aging population, and as such is expected to represent an increasingly important public health problem in our country. Thus, support from the national health authorities and national health funding system is essential in wider application of this novel technique, as TAVI is therapeutic option that has demonstrated improved survival and quality of life. Target population for TAVR includes symptomatic patients with severe aortic stenosis who are refused by the surgeons due to high surgical risk, and the estimated percentage is around 0.02% of the whole population [1]. Thus, for the population of our country the estimate would be around 150 patients *per* year which is far more than initially expected but appeared to be quite true as the number of patients with AS are either not diagnosed at all or underdiagnosed and poorly referred to the surgeons. Thus, with initiation of TAVI program in other countries, the number of surgical procedures for aortic valve replacement did not decrease, but on the contrary increased due to higher awareness of this severe disease and better referral for the treatment.

Complications of TAVI include stroke in 1–5% of the patients [1–4], onset of new left bundle branch block (in 7–18% with balloon-expanding valves and in 30–80% of patients with self-expanding valves) [13], need for new pacemaker (in 7–17.3% for the balloon expanding valves and in 37–40% for the self-expanding valves) [2, 14, 15], and vascular complications up to 20%, [2, 3, 16]. Also, trace or mild paravalvular regurgitation is a common finding in the majority of the patients after TAVI procedure [1]. However more than a mild paravalvular regurgitation may have an impact on long-term survival of the patients [17, 18]. None of our first 5 patients developed any of the common TAVI complications.

The first mechanical heart valve on mitral position was surgically implanted in March 1960 [19]. Next year Harken et al. [20] reported a small series with caged-ball prosthesis in the aortic position, and 40 years later Alain Cribier implanted first transcatheter aortic valve by anterograde approach [2, 21]. In 2004, the first pure retrograde approach was performed, and the first complete percutaneous retrograde implantation of CoreValve (Medtronic, USA) was done in 2006 [2, 21]. Until today, more than 90% of cases were performed either with self-expandable CoreValve (Medtronic) or balloon-expandable Edwards/Sapien aortic valve, but more than 10 different aortic valves with different technical characteristics and designs are under clinical investigations. Survival rates after CoreValve implantation is excellent and range from 71–84% after 1 year in more than 500 patients [5, 16, 17]. Similarly, PARTNER trial, the first randomized trial on AS in non-surgical candidates, demonstrated significant reduction in all cause and cardiovascular death in patients treated with TAVI in comparison to the standard therapy [2, 22]. In addition, excellent worldwide experience and safety profile with TAVI in high risk patients, in 2014 further lowers the threshold for this procedure to patients with moderate surgical risk [23].

Conclusion

Our initial results with CoreValve transcatheter aortic valve implantation (TAVI) is promising and encouraging, improvement of symptoms and hemodynamics excellent, and this initial experience opens the therapeutic door to treat a large number of patients who remain to be treated.

Acknowledgment

This research was supported with the grant by Ministry of Education, Science and Technologycal Development of the Republic of Serbia under research project number ON 175 020.

REFERENCES

1. *Vahanian A, Alfieri O, Andreotti F, Antunes MJ, Barón-Esquivias G, Baumgartner H,* et al. Guidelines on the management of valvular heart disease (version 2012). Eur Heart J 2012; 33(19): 2451−96.
2. *Leon MB, Smith CR, Mack M, Miller CD, Moses JW, Svensson LG,* et al. Transcatheter aortic-valve implantation for aortic stenosis in patients who cannot undergo surgery. N Engl J Med 2010; 363(17): 1597−607.
3. *Généreux P, Head SJ, Wood DA, Kodali SK, Williams MR, Paradis J,* et al. Transcatheter aortic valve implantation 10-year anniversary: review of current evidence and clinical implications. Eur Heart J 2012; 33(19): 2388−98.
4. *Athappan G, Gajulapalli R, Sengodan P, Bhardwaj A, Ellis SG, Svensson L,* et al. Influence of transcatheter aortic valve replacement strategy and valve design on stroke after transcatheter aortic valve replacement: a meta-analysis and systematic review of literature. J Am Coll Cardiol 2014; 63(20): 2101−10.
5. *Ussia GP, Barbanti M, Petronio AS, Tarantini G, Ettori F, Colombo A,* et al. Transcatheter aortic valve implantation: 3-year outcomes of self-expanding CoreValve prosthesis. Eur Heart J 2012; 33(8): 969−76.

6. *Moat NE, Ludman P, de Belder MA, Bridgewater B, Cunningham AD, Young CP,* et al. Long-term outcomes after transcatheter aortic valve implantation in high-risk patients with severe aortic stenosis: the U.K. TAVI (United Kingdom Transcatheter Aortic Valve Implantation) Registry. J Am Coll Cardiol 2011; 58(20): 2130−8.
7. *Tchetche D, Dumonteil N, Sauguet A, Descoutures F, Luz A, Garcia O,* et al. Thirty-day outcome and vascular complications after transarterial aortic valve implantation using both Edwards Sapien and Medtronic CoreValve bioprostheses in a mixed population. EuroIntervention 2010; 5(6): 659−65.
8. *Martínez GJ, Seco M, Jaijee SK, Adams MR, Cartwright BL, Forrest P*, et al. Introduction of an interdisciplinary heart team-based transcatheter aortic valve implantation programme: short and mid-term outcomes. Intern Med J 2014; 44(9): 876−83.
9. *Davies WR, Thomas MR*. European experience and perspectives on transcatheter aortic valve replacement. Prog Cardiovasc Dis 2014; 56(6): 625−34.
10. *Giordana F, D'Ascenzo F, Nijhoff F, Moretti C, D'Amico M, Biondi ZG,* et al. Meta-analysis of predictors of all-cause mortality after transcatheter aortic valve implantation. Am J Cardiol 2014; 114(9): 1447−55.
11. *Zajarias A, Cribier AG*. Outcomes and safety of percutaneous aortic valve replacement. J Am Coll Cardiol 2009; 53(20): 1829−36.
12. *Nedeljkovic M, Vukcevic V, Beleslin B, Stankovic G, Tesic M, Stojkovic S*, et al. First transcatheter implantations of aortic valve in Serbia. Srce i krvni sudovi 2014; 33(1): 30−4. (Serbian)
13. *Agarwal S, Tuzcu EM, Krishnaswamy A, Schoenhagen P, Stewart WJ, Svensson LG,*et al. Transcatheter aortic valve replacement: current perspectives and future implications. Heart 2015; 101(3): 169–77.
14. *Abdel-Wahab M, Mehilli J, Frerker C, Neumann F, Kurz T, Tölg R,* et al. Comparison of balloon-expandable vs self-expandable valves in patients undergoing transcatheter aortic valve replacement: the CHOICE randomized clinical trial. JAMA 2014; 311(15): 1503−14.
15. *Zahn R, Gerckens U, Grube E, Linke A, Sievert H, Eggebrecht H,* et al. Transcatheter aortic valve implantation: first results from a multi-centre real-world registry. Eur Heart J 2011; 32(2): 198−204.
16. *Chiam PT, Ewe SH*. An update on complications associated with transcatheter aortic valve implantation: stroke, paravalvular leak, atrioventricular block and perforation. Future Cardiol 2013; 9(5): 733−47.
17. *Zahn R, Gerckens U, Linke A, Sievert H, Kahlert P, Hambrecht R,* et al. Predictors of one-year mortality after transcatheter aortic valve implantation for severe symptomatic aortic stenosis. Am J Cardiol 2013; 112(2): 272−9.
18. *Tamburino C, Capodanno D, Ramondo A, Petronio AS, Ettori F, Santoro G,* et al. Incidence and predictors of early and late mortality after transcatheter aortic valve implantation in 663 patients with severe aortic stenosis. Circulation 2011; 123(3): 299−308.
19. *Edmunds LH*. Evolution of prosthetic heart valves. Am Heart J 2001; 141(5): 849−55.
20. *Harken DE, Soroll HS, Taylor WJ*, et al. Aortic valve replacement. In: *Meredino KA,* editor. Prosthetic valves for cardiac surgery. Springfild (IL): Charles C Thomas; 1961. p. 508–25.
21. *Cribier A*. Historical perspective: 10th year anniversary of TAVI. EuroIntervention. 2012; 8(Suppl Q): Q15−7.
22. *Kodali SK, Williams MR, Smith CR, Svensson LG, Webb JG, Makkar RR,* et al. Two-year outcomes after transcatheter or surgical aortic-valve replacement. N Engl J Med 2012; 366(18): 1686−95.
23. *Cribier A, Durand E, Eltchaninoff H*. Patient selection for TAVI in 2014: is it justified to treat low- or intermediate-risk patients? The cardiologist's view. EuroIntervention 2014; 10 Suppl U: U16−21.

Received on November 17, 2014.
Revised December 14, 2014.
Accepted on December 19, 2014.
Online First April, 2015.

C A S E R E P O R T S

UDC: 340.6:616-091.5-074
DOI: 10.2298/VSP141105022B

Fatal cocaine intoxication in a body packer

Letalno trovanje kokainom kod *body packer*-a

Gordana Brajković*, Gordana Babić*, Jasna Jović Stošić*†, Gordana Tomašević‡, Dragana Rančić*, Vesna Kilibarda*†

***National Poison Control Centre, ‡Forensic Medicine and Pathology, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defence, Belgrade, Serbia**

Abstract

Introduction. 'Body packer' syndrome with severe intoxication or sudden death may happen in persons who smuggle drugs in their body cavities. In case of lethal outcome when carrying cocaine, it is important, but sometimes difficult to determine whether death was due to intoxication or due to other causes. Therefore, it is necessary not only to quantify cocaine and its metabolites in biological material, but also based on their distribution in body fluids and tissues to conclude whether it is acute intoxication. We described a well-documented case of fatal poisoning in a body packer and *post mortem* distribution of the drug in biological samples. **Case report.** A 26-year-old man was brought to hospital with no vital signs. Resuscitation measures started at once, but with no success. Autopsy revealed 66 packets of cocaine in his digestive tract, one of which was ruptured. Hyperemia of the most of all internal organs and pulmonary and brain edema were found. High concentrations of cocaine, its metabolites benzoylecgonine and ecgonine methyl ester, as well as cocaine adulteration levamisole were proven in the *post mortem* blood and tissues by liquid chromatography-mass spectrometry (LC-MC) method with selective-ion monitoring. **Conclusion.** The ratio of cocaine and its metabolites concentrations in the brain and blood obtained by LC-MS method can be used for forensic confirmation of acute intoxication with cocaine.

Key words:
cocaine; poisoning; deglutition; death, sudden; diagnosis, differential; chromatography.

Apstrakt

Uvod. Kod osoba koje krijumčare drogu u svojim telesnim šupljinama može doći do pojave sindroma *body packer*, odnosno teškog trovanja, ili iznenadne smrti. U slučaju letalnog ishoda prilikom prenošenja kokaina, važno je, ali ponekad i jako teško, utvrditi da li je smrt nastupila usled intoksikacije. Zbog toga je značajno ne samo kvantifikovati kokain i njegove metabolite u biološkom materijalu, već i na osnovu njihove raspodele u telesnim tečnostima i tkivima zaključiti da li se radi o akutnoj intoksikaciji. Opisali smo dobro dokumentovani slučaj letalnog trovanja kod *body packer*-a i *post mortem* raspodelu droge u biološkim uzorcima. **Prikaz bolesnika.** Kod 26-godišnjeg mladića neposredno pre prijema u bolnicu došlo je do prestanka vitalnih funkcija. Na prijemu je pokušana reanimacija, koja je ostala bez odgovora. Prilikom obdukcije u gastrointestinalnom traktu nađeno je 66 paketića kokaina, od kojih je jedan ruptuirao. Patološkim pregledom otkrivena je hiperemija unutrašnjih organa, edem pluća i mozga. Primenom metode tečne hromatografije sa masenom spektrometrijom (LC-MS) u *post mortem* biološkom materijalu dokazane su visoke koncentracije kokaina, njegovih metabolita benzoilekgonina i ekgonin-metil-estra, kao i levamisola koji predstavlja dodatak kokainu. **Zaključak.** Odnos konentracija kokaina i njegovih metabolita u mozgu i krvi koje su dobijene LC-MS metodom može se koristiti radi forenzičke potvrde akutne intoksikacije kokainom.

Ključne reči:
kokain; trovanje; gutanje; smrt, iznenadna; dijagnoza, diferencijalna; hromatografija.

Introduction

Body packers are individuals who transport illicit drugs, usually cocaine, heroin, or amphetamines in their bodies. This is a well-known method of smuggling illegal substances, which are packed into condoms or double latex balloons and then ingested in large numbers [1]. Each individual packet contains a lethal quantity of narcotics. Sometimes intestinal obstruction may occur, but more dangerous is bursting of a packet with leakage of the content which may be rapidly fatal. These consequences are known as a "body packer" syndrome [2].

The Republic of Serbia is a part of the Balkan corridor and a transit country for narcotics. One of the well-known drug trafficking routes leads from Turkey and Afghanistan, through Ser-

Correspondence to: Gordana Brajković, National Poison Control Center, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. Phone.: +381 11 3609 481. E-mail: gordanatox@gmail.com

bia, to Central and Western Europe [3,4]. However, cocaine smuggling is usually conducted by air transport from South America (Brazil, Argentina, Uruguay), the Caribbean Islands (The Dominican Republic, Jamaica) and Central America (Costa Rica), and body packing is the method to do it.

Cocaine is most often taken intranasally (by snorting), or injected intravenously, but other routes are also possible, including oral (by chewing), inhalation (smoking), intravaginal and rectal application. The mucosal absorption of cocaine, in comparison to the intravenous one, achieves the maximum effect at a later time, but lasts longer [5].

Cocaine is metabolized through enzyme and non-enzyme hydrolysis to benzoylecgonine. Ecgoninemethyl ester, the second major metabolite of cocaine, is created by a hydrolytic effect of cholinesterases [6] .

The toxic effects of cocaine usually appear when the concentrations of 0.25 to 0.5 mg/L are present in blood. Lethal outcomes have been recorded with the concentrations of 1.0 mg/L [7].

Cocaine is unstable in *post mortem* samples due to its degradation and redistribution. Spiehler and Reed [8], and later Kalasinsky et al. [9], demonstrated that brain tissue is probably the most valuable sample for determining the concentration of cocaine and its metabolites, due to their uniform distribution in brain tissue, stability after freezing, and the absence of post mortem redistribution.

In case of lethal outcome when carrying cocaine, it is important, but sometimes difficult to determine whether the death was due to intoxication or due to other causes. Therefore, it is necessary not only to quantify cocaine and its metabolites in biological material, but also based on their distribution in body fluids and tissues to conclude whether it is acute intoxication. We described a well-documented case of fatal poisoning in a body packer and *post mortem* distribution of the drug in biological samples suggesting severe acute cocaine intoxication.

Case report

A 26-year-old man arrived at Belgrade Airport from the Dominican Republic. He was feeling seek, so left at the entrance of Emergency Department of the Military Medical Academy (MMA). At admission, the patient was without vital signs. Cardiopulmonary resuscitation (CPR) was initiated immediately, but with no response. A blood sample was taken for toxicological analysis during CPR. Analysis revealed the presence of high concentrations of cocaine (5.19 mg/L), its metabolites benzoylecgonine (3.1 mg/L) and ecgonine methyl ester (8.77 mg/L), as well as levamisole (1.57 mg/L).

Autopsy was performed due to "sudden" death. Sixty-six identical oval-shaped packets were retrieved from the body, with 3 packets being found in the stomach and the rest 63 in the intestines (Figure 1). One of them was ruptured. The overall weight of the contents was 700 g.

Analysis of one packet content was done, the remaining 65 packets were handed over to the police. The delivered packet was oval, 1.7 × 4.5 cm in diameter, containing 10 g of white powder densely packed into a latex sheath, similar to the condom. It was tied at the open end, covered with several other layers of latex, and sealed with a hard wax coating. The outer layer consisted of a plastic bag. The remaining drug packets were of the same size and consistency, which suggested an automated process of packaging. Figure 2 shows a drug packet recovered from the patient. Cocaine powder was of 66.8% purity and contained levamisole as an adulterant.

Fig. 1 – Outlines of several oval-shaped drug packets can be seen in the intestines of the patient.

Fig. 2 – Drug packet recovered from the patient.

An autopsy exhibited hyperaemia in almost all of the patient's internal organs, including brain, heart, lung, kidney, spleen, liver and adrenal glands. Brain edema and pulmonary edema were also present.

Toxicological analysis confirmed the presence of cocaine, its metabolites and levamisole in the patient's internal organs, blood and urine samples.

The extracts of post mortem fluids (2 mL) and tissue specimens (2 g homogenate), were analyzed by liquid chromatography–mass spectrometry CLS-MS using with XTerra column, mobile phase ammonium formate: acetonitrile 5mM (pH 3.5) in the gradient mode. The ESI was operated in positive mode for the detection of cocaine, benzoylecgonine, ecgonine methyl ester and levamisole at the following mass to charge rations (m/z): cocaine m/z 304→182, benzoylecgonine m/z 290 → 168, ecgonine methyl ester m/z 200→182, levamisole m/z 205 →178. Quantification of the substance in the samples was made

using a 5-point calibration curve, a validated method for cocaine, benzoylecgonine, ecgonine methyl ester and levamisole. The results of the *post mortem* toxicological analyses are shown in Table 1.

Table 1

Results of toxicological analyses in post mortem samples

Sample	Concentration (mg/L) (mg/kg*)			
	Cocaine	Benzoylecgonine	Ecgonine methyl ester	Levamisole
Blood	4.06	10.42	18.77	1.91
Urine	71.88	684.72	791.67	52.32
Liver with gall bladder	21.21	31.43	71.71	17.37
Kidney and bladder	24.93	16.00	30.91	20.54
Brain*	18.95	1. 95	11.37	6.90
Heart, lung, spleen	9.21	8.43	15.96	8.04
Stomach	4.46	6.35	12.50	2.13
Small and large intestine	6.11	27.76	39.16	2.94

Discussion

We described probably the first case of body packer's death in Serbia caused by cocaine poisoning which was proved by autopsy and toxicological analyses (LC-MS method). The fatal intoxication occurred due to a leakage of one packet's content into the gastrointestinal tract. Analysis of the white powder retrieved from the packet confirmed the presence of cocaine and levamisole. Levamisole has been identified as a cocaine adulterant for more than a decade [10]. In 2010 the US Drug Enforcement Administration (DEA) reported that 79% of all cocaine seized in the United States contained levamisole [11]. The mass fraction of levamisole in cocaine samples increased during the time, reaching about 50% or more in recent years in some countries [12]. Our sample contained about 2/3 of cocaine and 1/3 of levamisole. Several potential mechanisms are proposed to explain why this particular drug is added to cocaine. Nicotinic effect of levamisole may result in increased release of norepinephrine and dopamine, enhancing sympathomimetic activity and elevating mood. Recently published study confirmed the metabolism of levamisole to the amphetamine-like compound aminorex, which may add to stimulating effect of cocaine [13].

No safe concentration of cocaine in blood has been defined, but concentration greater than 5 mg/L usually results in severe intoxication or death [14]. In our case, concentration of cocaine in the sample obtained during resuscitation was 5.19 mg/L.

Cocaine rapidly metabolizes in the body to two major metabolites, benzoylecgonine and, ecgonine methylester, and several minor metabolites [15]. As a result of hydrolysis in blood, concentration determined *post mortem* may not reflect the actual concentration in the body at the time of death. Accordingly, concentration of cocaine and its metabolites in brain tissue samples are important for interpreting the results. Benzoylecgonine does not cross the blood-brain barrier and it can be found in the brain only if it was formed there. Having in mind the fact that at the moment of death blood flow and metabolism stop, relative amounts of cocaine and benzoylecgonine could be used for drawing conclusions whether it is a case of acute or chronic exposure to cocaine. A high concentration of cocaine with a low or non-existent concentration of benzoylecgonine indicates acute poisoning. The presence of benzoylecgonine, without a parent compound in brain tissue, indicates the exposure that was a long time ago [16]. We have determined high concentration of cocaine (18.95 mg/kg) and much lower concentration of benzoylecgonine (1.95 mg/kg) in brain tissue sample suggesting acute overdose.

As cocaine readily crosses the blood-brain barrier and benzoylecgonine does not, in acute overdoses brain concentrations of cocaine may be several times higher, and of benzoylecgonine several times lower than those in plasma [8]. In the presented case, the brain/blood ratio for cocaine was approximately 4.67 and for benzoylecgonine was 0.15.

The highest cocaine concentrations were found in urine and the kidney, followed by the concentrations in the brain and the liver. These results are in agreement with previously reported tissue disposition of cocaine in acute fatal poisonings [17].

Conclusion

Significant morbidity and mortality in body 'packer' syndrome are the direct results of cocaine absorption from the gastrointestinal tract. From the point of view of forensic toxicology analysis, isolated cocaine blood levels are not sufficient for the diagnosis of cause of death. They should always be considered and evaluated in relation to concentrations of cocaine and benzoylecgonine in body tissue compartments, especially in the brain. Brain tissue is a useful specimen for determination of cocaine and its metabolites, because concentrations are relatively stable and do not undergo *post mortem* redistribution. Accordingly, the ratio between cocaine and benzoylecgonine in the brain, as well as relative amounts of these substances in the brain and blood may indicate acute overdose.

R E F E R E N C E S

1. *Pidoto RR, Agliata AM, Bertolini R, Mainini A, Rossi G, Giani G.* A new method of packaging cocaine for international traffic and implications for the management of cocaine body packers. J Emerg Med 2002; 23(2): 149−53.
2. *McCarron MM, Wood JD.* The cocaine 'body packer' syndrome. Diagnosis and treatment. JAMA 1983; 250(11): 1417−20.
3. United Nations Office on Drugs and Crime (UNODC). World drug report 2010. In: Publication Sales. Vienna: United Nations. 2010. No.E.10.XI.13; 83−5. Available from: http://www.unodc.org/documents/wdr/WDR_2010/World_Drug_Report_2010_lo-res.pdf.
4. Drug and Chemical Control; International Narcotics Control Strategy Report INCSR. Washington DC: United States Department of State; Bureau for International Narcotics and Law Enforcement Affairs; 2012. Available from: http://www.state.gov/documents/organization/184314.pdf.
5. *Egred M, Davis GK.* Cocaine and the heart. Postgrad Med J 2005; 81(959): 568−71.
6. *Heard K, Palmer R, Zahniser NR.* Mechanisms of acute cocaine toxicity. Open Pharmacol J 2008; 2(9): 70−8.
7. *Moffat AC, Osselton DM, Widdop B.* Clarke's Analysis of Drugs and Poisons in pharmaceuticals, body fluids and posmortem material, 3rd ed. London: Pharmaceutical press; 2004. p. 687−8; 842−5; 1168; 1614.
8. *Spiehler VR, Reed D.* Brain concentrations of cocaine and benzoylecgonine in fatal cases. J Forensic Sci 1985; 30(4): 1003−11.
9. *Kalasinsky KS, Bosy TZ, Schmunk GA, Ang L, Adams V, Gore SB,* et al. Regional distribution of cocaine in postmortem brain of chronic human cocaine users. J. Forensic Sci 2000; 45(5): 1041−8.
10. *Larocque A, Hoffman RS.* Levamisole in cocaine: Unexpected news from an old acquaintance. Clin Toxicol (Phila) 2012; 50(4): 231−41.
11. *Bertol E, Mari F, di Milia MG, Politi L, Furlanetto S, Karch SB.* Determination of aminorex in human urine samples by GC–MS after use of levamisole. J Pharm Biomed Anal 2011; 55(5): 1186−9.
12. *Schneider S, Meys F.* Analysis of illicit cocaine and heroin samples seized in Luxembourg from 2005–2010. Forensic Sci Int 2011; 212(1−3): 242−6.
13. *Hofmaier T, Luf A, Seddik A, Stockner T, Holy M, Freissmuth M,* et al. Aminorex, a metabolite of the cocaine adulterant levamisole, exerts amphetamine like actions at monoamine transporters. Neurochem Int 2014; 73(100): 32−41.
14. *Karch SB.* Introduction to the forensic pathology of cocaine. Am J Forensic Med Pathol 1991; 12(2): 126−31.
15. *Kolbrich EA, Barnes AJ, Gorelick DA, Boyd SJ, Cone EJ, Huestis MA.* Major and Minor Metabolites of Cocaine in Human Plasma following Controlled Subcutaneous Cocaine Administration. J Analyt Toxicol 2006; 30(8): 501−10.
16. *Stephens BG, Jentzen JM, Karch S, Mash DC, Wetli CV.* Criteria for the Interpretation of Cocaine Levels in Human Biological Samples and Their Relation to the Cause of Death. Am J Forensic Med Pathol 2004; 25(1): 1−10.
17. *Poklis A, Mackell MA, Graham M.* Disposition of Cocaine in Fatal Poisoning in Man. J Anal Toxicol 1985; 9(5): 227−9.

Received on November 5, 2014.
Revised on December 30, 2014.
Accepted on January 15, 2015.
Online First April, 2015.

CASE REPORT

UDC: 616-056.7:616.24
DOI: 10.2298/VSP141007146I

Lung parenchima changes in neurofibromatosis type 1

Promene parenhima pluća kod neurofibromatoze tipa 1

Aleksandra Ilić*†, Snežana Raljević*†, Tatjana Adžić*†, Vesna Škodrić-Trifunović*†, Jelena Stanimirović*

***Clinic for Lung Diseases, Clinical Center of Serbia, Belgrade, Serbia; †Faculty of Medicine, University of Belgrade, Belgrade, Serbia**

Abstract

Introduction. Neurofibromatosis type 1 (NF1), also known as von Recklinghausen disease, is one of the most common single-gene disorders (mutation on chromosome 17q) and usually associated with cutaneous, musculoskeletal and neurological disorders in humans. NF1 is generally complicated with one or more neurobehavioral disorders or tumors located in the peripheral nervous system such as neurofibromas, peripheral nerve sheath tumor, pheochromocytoma, etc. In the available medical literature, the thoracic manifestations of NF1 have been rarely described in these patients. There are few reports about intrathoracic neurogenic tumors, kyphoscoliosis, pneumonitis and pulmonary fibrosis in patients with NF1. **Case report.** A 65-year-old female was admitted to the Intensive Care Unit at the Lung Clinic of Belgrade University Clinical Center of Serbia. The patient's general condition was poor with shortness of breath and present cyanosis. At the same time, the skin changes similar to NF1 were noticed, which were additionally documented by her medical history and diagnosed as NF1. After the application of noninvasive mechanical ventilation and other emergency respiratory medicine measures, the patient soon felt better. The parenchymal changes were viewed by subsequent X-rays and CT scanning of the thorax. **Conclusion.** This is a case report presenting the NF1 associated with the abnormality of lung parenchyma established during diagnostic procedures at the Intensive Care Unit, Clinic of Pulmonology.

Key words:
neurofibromatoses; diagnosis; lung fibrosis; radiography; tomography, x-ray computed.

Apstrakt

Uvod. Neurofibromatoza tipa 1 (NF1), takođe poznata kao fon Recklinghauzenova bolest, jedan od najčešćih poremećaja pojedinačnih gena (mutacija na hromozomu 17q), obično je povezana sa kožnim, mišićnoskeletnim i neurološkim poremećajima kod ljudi. Takođe, NF1 obično je povezana sa jednim ili više neurobihejvioralnih poremećaja ili tumora lociranih na perifernom nervnom sistemu, kao što su neurofibromi, plašt tumori perifernih nerava, feohromocitom, itd. Kod istih bolesnika torakalne manifestacije NF1 opisane su retko u sadašnjoj medicinskoj literaturi. Postoji nekoliko izveštaja o intratorakalnim neurogenskim tumorima, kifoskoliozi, pneumonitisu i plućnoj fibrozi kod bolesnika sa NF1. **Prikaz bolesnika.** Bolesnica, stara 65 godina, na prijemu u Jedinicu intenzivne nege Klinike za pulmologiju Kliničkog centra Srbije bila je u lošem opštem stanju, kratakog daha i sa prisutnom cijanozom. U tom trenutku primećene su i promene na koži. Nakon dobijanja informacije kao i podataka iz medicinske istorije bolesnice, utvrđeno je da je u pitanju NF1. Posle primene neinvazivne mehaničke ventilacije i drugih mera hitne respiratorne medicine, bolesnica se ubrzo osećala bolje. Na kasnije učinjenom RTG snimku i CT skenu grudnog koša uočene su promene parenhima. **Zaključak.** U ovom prikazu opisana je NF1 povezana sa abnormalnostima plućnog parenhima, utvrđena tokom dijagnostičkih procedura u Jedinici intenzivne nege Klinike za pulmologiju.

Ključne reči:
neurofibromatoza; dijagnoza; pluća, fibroza; radiografija; tomografija, kompjuterizovana, rendgenska.

Introduction

Neurofibromatosis belongs to the group of diseases called phakomatoses. Neurofibromatosis type 1 (NF1) is caused by gene mutation on chromosome 17q which encodes a protein known as neurofibromin, a negative regulator of Ras oncogene [1]. NF1 has a number of possible signs. The presence of *café-au-lait* maculas and multiple neurofibromas, usually 5–15 mm in diameter was a key sign of diagnosis [2]. Chest manifestations may be different: kyphoscoliosis, ribbon deformity of the ribs, intrathoracic neoplasms, and interstitial lung disease such as diffuse interstitial fibrosis and bullous lung disease either alone or in combination, sometimes called fibrosing alveolitis [3].

Correspondence to: Jelena Stanimirović, Intesive Care Unit, Clinic for Lung Diseases, Clinical Center of Serbia, Koste Todorovića 26, 11 000 Belgrade, Serbia. Phone: +381 11 366 3457. E-mail: j.stanimirovic@hotmail.com

Case report

A 65-year-old woman was admitted to the Intensive Care Unit, Clinic of Pulmonology, University Clinical Center of Serbia, Belgrade, due to crisis of consciousness, shortness of breath and very pronounced cyanosis. The patient's medical history, dating back as far as 30 years, showed that the patient had been treating for chronic obstructive pulmonary disease (COPD) all the time. The last few years, the patient experienced more frequent exacerbations.

Moreover, the patient had manifested NF1 disease since childhood in the form of *café-au- lait* maculae and multiple neurofibromas covering almost the entire skin; she had also had positive family medical history of some manifestations of the disease presented in her mother and grandfather. Since 2011, the patient had been suffering from verified tachyarrhythmia, high blood pressure and chronic cardiomyopathia. During that period, thoracic computed tomography (CT) scanning revealed, for the first time, abundant sequelae of specific process and fibrothorax in the left lung. The patient was skinny with almost child constitution: height of 152 cm, weight 45 kg, body mass index (BMI) 19.48 kg/m^2. She was a non smoker. Physical examination showed multiple maculae on the entire skin (Figure 1) and deformity of the spine in the form of kyphoscoliosis. Respiratory findings were as follows: lower airway sounds and polyphonic wheezing. Cardiac findings were: tachycardia, and cardiac arrhythmic action with occasional extrasystoles; blood pressure: 130/100 mmHg; heart rate: 121/min. Abdomen and limbs were clinically normal. Laboratory analysis showed mild positive inflammatory syndrome, anemia and thrombocytopenia, hypoproteinemia and electrolyte imbalance. Arterial blood gas analyses were in favor of acidosis and global respiratory insufficiency. Spirometry results were: forced vital capacity (FVC) 1.23 L (56%); predicted forced expiratory volume in 1 second (FEV1) 0.67 L (37%); predicted ratio FEV1/FVC 54.92%; mixed ventilatory pattern, dominantly obstructive and reduced diffusing capacity of the lung; diffusing capacity of the lung for carbon monoxide (DLCO) 31%; transfer coefficient (KCO) 54%. The chest x-ray showed an increased transparency from the apex to the base, pleuropericardial and pleurobasal adhesions, with fibrous changes in parenchyma on both sides (Figure 2). Computed tomography scan revealed solid emphysema and bullous changes viewed in the apical left, right and posterior areas (Figure 3). Fibroindurative chronic changes with the interstitial inflammation and hilar traction were observed in bilateral the upper medial lobes. Bullous changes with bronchiectasis were present basally on the left side. Paratracheal lymph nodes, 18 mm in diameter, were also evident. Bilateral pleural effusion was basally organized. Diffuse lung reduction was recorded (Figure 4).

Fig. 1 – Physical examination showed multiple *maculae* on the entire skin.

Fig. 2 – Chest x-ray showed on both sides an increased transparency from the apex to the base as well as pleuropericardiac and pleurobasal adhesions, with fibrous changes of parenchyma.

Fig. 3 – CT scanning showing apical left, right and back solid emphysema and bullous changes.

Fig. 4 – CT scanning showing bullous changes with bronchiectasis to the basal left. paratracheal lymph nodes 18 mm in diameter, bilateral pleural effusion, basal, organized, as well as diffuse lung reduction.

Discussion

The condition of the presented patient fully fits in the clinical picture of NF1. At least two criteria have to be recognized for establishing the diagnosis of NF1: the presence of minimum six *cafe-au-lait* spots of more than 5 mm in diameter in children and 15 mm in adults; no less than two neurofibromas or one plexiform neurofibroma; freckles and/or discoloration in the sun-protected areas (armpits, pubic area); optic glioma; the presence of at least two iris nodules (Lisch nodules); specific skeletal abnormalities; hereditary high blood pressure [4]. Appropriate diagnostic procedures confirmed the presence of lung changes. Although pulmonary fibrosis, bullae or other interstitial abnormalities are uncommon findings with neurofibromatosis, the parenchyma lung involvement has been reported in 1.9–20% of NF1. The first cases of chest abnormalities in NF1 were described in 1963 [5]. Some studies show that the increased sensitivity of the lung to cigarette smoke causes an early development of emphysema-like changes [5, 6]. Since the identification of the NF1 gene, great advances in understanding the role of the NF1 gene in molecular pathogenesis of NF1-associated clinical abnormalities have been achieved [5]. Clinical studies have begun to define specific subpopulations of patients at risk for cancer and have identified targeted therapies for NF1-associated tumors, based on science research advances [7]. Quality of life in NF1may be different and it depends on many factors: sex, age, emotions, physical symptoms, functioning, and other associated diseases. Patients with more severe NF1 reported more effects on the physical function, general health perception and vitality [8]. Patients with neurofibromatosis and respiratory symptoms need to be checked for possible changes in the lung parenchyma, and they require a multidisciplinary approach [1, 9].

Conclusion

We presented a patient, non-smoker, whose changes in the lung parenchyma were most likely not caused by COPD for which the patient was treated over 30 years. The impairment of pulmonary function was the result of kyphoscoliosis and emphysema changes in the lung parenchyma. Only a few available references on the association between NF1 and changes in pulmonary parenchyma indicate that this problem has been rarely recorded. Well documented NF1 case reports indicate that the routine tests (X-ray, CT scan, spirometry, diffusion) can detect pulmonary changes in the early stages of the disease, which is certainly important for better prognosis.

REFERENCES

1. *Viskochil D, Buchberg AM, Xu G, Cawthon RM, Stevens J, Wolff RK,* et al. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. Cell 1990; 62(1): 187−92.
2. *Huson SM.* The neurofibromatosis: classification, clinical features and genetic counselling. In: *Kaufmann D,* editor. Neurofibromatoses. Monogr Hum Genet. Manchester (UK): Basel & Karger; 2008; 16: 1−20.
3. *Aughenbaugh GL.* Thoracic manifestations of neurocutaneous diseases. Radiol Clin North Am 1984; 22(3): 741−56.
4. *Nelepa P, Wolnicka M.* Neurofibromatosis type 1 with interstitial pulmonary lesions diagnosed in an adult patient. Pneumonol Allergol Pol 2012; 80(2): 152−7.
5. *Massaro D, Katz S.* Fibrosing alveolitis: its occurrence, roentgenographic, and pathologic features in von Recklinghausen's neurofibromatosis. Am Rev Respir Dis 1966; 93(6): 934−42.
6. *Davies PD.* Diffuse pulmonary involvement in Von Recklinghausen's disease: a new syndrome. Thorax 1963; 18: 198.
7. *Arun D, Gutmann DH.* Recent advances in neurofibromatosis type 1. Curr Opin Neurol 2004; 17(2): 101−5.
8. *Wolkenstein P, Zeller J, Revuz J, Ecosse E, Leplège A.* Quality-of-life impairment in neurofibromatosis type 1: a cross-sectional study of 128 cases. Arch Dermatol 2001; 137(11): 1421−5.
9. *Ferner RE, Huson SM, Thomas N, Moss C, Willshaw H, Evans D,* et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. J Med Genet 2007; 44(2): 81−8.

Received on October 7, 2014.
Revised on January 6, 2015.
Accepted on February 5, 2015.
Online First December, 2015.

CASE REPORT

UDC: 617.55::616.681-006-007.41-08
DOI: 10.2298/VSP150126021J

Giant primary retroperitoneal seminoma: A case report

Veliki primarni retroperitonealni seminom

Milan Jovanović*†, Nataša Janjušević†, Darko Mirković*†, Maja Vulović‡, Boško Milev*, Miroslav Mitrović*, Bratislav Trifunović*†

***Clinic for General Surgery, Military Medical Academy, Belgrade, Serbia; †Faculty of Medicine of the Military Medical Academy, University of Defense, Belgrade, Serbia; ‡Department of Anatomy and Forensic Medicine, Faculty of Medical Sciences, University of Kragujevac, Kragujevac, Serbia**

Abstract

Introduction. Primary extragonadal seminomas are rare tumors. There have been only a few cases of the primary retroperitoneal seminomas reported in the literature up to date. **Case report.** We reported a 56-year-old man with giant primary retroperitoneal seminoma presented with the enlargement of the left side of the abdomen and deep venous thrombosis of the left leg. Computed tomography of the abdomen showed a large tumor occupying the left part of the retroperitoneal space with 23 × 13 cm in diameter. Firm tumor mass having 25 × 15 cm in diameter was surgically removed from the left retroperitoneum. The tumor adhered the *tunica adventitia* of the aorta and it was carefully resected from the aortic wall. The diagnosis of seminoma was made during histopathological examination. The patient underwent chemotherapy. Two years after finished chemotherapy the patient accepted left orchiectomy with the aim of eliminating the possibility of the occult malignancy of the testicle. Histopathological analysis of the testicular tissue was normal and the diagnosis of primary retroperitoneal seminoma was confirmed. **Conclusion.** Despite its small incidence in general population, the diagnosis of retroperitoneal seminoma should be considered in male patients with nonspecific symptoms and with retroperitoneal tumor mass.

Key words:
seminoma; retroperitoneal space; venous thrombosis; diagnosis, differential; digestive system surgical procedures; orchiectomy.

Apstrakt

Uvod. Primarni vantestisni seminomi su veoma retki tumori. U literaturi je prikazano svega nekoliko slučajeva primarnog retroperitonealnog seminoma. **Prikaz bolesnika.** Prikazali smo bolesnika, starog 56 godina, sa ogromnim primarnim retroperitonealnim seminomom, koji se javio sa uvećanjem leve polovine abdomena i dubokom venskom trombozom leve noge. Na kompjuterizovanoj tomografiji abdomena opisan je tumor veličine 23 × 13 cm. Hirurškim putem uklonjena je čvrsta tumorska masa veličine 25 × 15 cm. Tumor se prilepio za tuniku adventiciju aorte, a pažljivom resekcijom odvojen je od zida aorte. Patohistološki pregled pokazao je da se radi o seminomu. Bolesnik je primio hemioterapiju. Dve godine nakon završene hemioterapije prihvatio je hirurško uklanjanje levog testisa da bi se eliminisala mogućnost postojanja skrivenog malignog tumora testisa. Patohistološkom analizom tkiva testisa nije ustanovljeno prisustvo tumora, tako da je potvrđena dijagnoza primarnog retroperitonealnog seminoma. **Zaključak.** Kod bolesnika muškog pola sa nespecifičnom simptomatologijom i retroperitonealnom tumorskom masom treba misliti i na dijagnozu primarnog retroperitonealnog seminoma uprkos njegovoj maloj incidenciji u opštoj populaciji.

Ključne reči:
seminom; retroperitonealni prostor; tromboza, venska; dijagnoza, diferencijalna; hirurgija digestivnog sistema, procedure; orhiektomija.

Introduction

Testicular cancers are rare tumors in the general population accounting for almost 1% of all cancers in men [1]. Among them, germ cell tumors are the most common malignancies that typically arise in the testicle. However, these tumors could primary develop extragonadaly in 1–2% of all seminomas [2]. There have been only a few cases of the primary retroperitoneal and mediastinal seminomas reported in the literature up to date [3]. In this paper we presented a patient with a rare giant primary retroperitoneal seminoma with the enlargement of the left side of the abdomen and deep venous thrombosis of the left leg.

Correspondence to: Milan Jovanović, Clinic for General Surgery, Military Medical Academy, Crnotravska 17, 11 000 Belgrade, Serbia. Phone: + 381 62 264 140. E-mail: mibine@gmail.com

Case report

A 56-year-old male was admitted to the Medical Military Academy due to suspected deep venous thrombosis of the left leg. The patient complained pain and the swelling of the left leg and temperature of 38.4°C. He also reported painless gradual swelling of the left half of the abdomen during the last two years, which become more severe accompanying with the sudden onset of pain that irradiated into the left lumbar region. Physical examination revealed a firm palpable mass in the left abdominal quadrants. Ultrasound examination confirmed a deep venous thrombosis in the left leg and a solid tumor mass in the abdomen, with no detectable pathological changes in the abdominal organs. Computed tomography (CT) of the abdomen with contrast enhancement showed a large tumor occupying the left part of the retroperitoneal space with 23 × 13 cm in diameter (Figure 1a, b). The tumor expanded beneath the diaphragm in front of the spine, spreading downward to the level of the inferior pole of the left kidney. The kidney was displaced cranially showing no signs of hydronephrosis, while the renal vein was partially compressed by the mass. Lower part of the tumor partially compressed the left iliac blood vessels. The tumor was in close relationship with the abdominal aorta that was also dislocated to the right (Figure 1c). Infiltration of the aortic wall in the length of 2 cm above the bifurcation by the mass was suspected. The patient underwent surgical therapy.

gonadotropin – β-HCG), that were not elevated. The testicles showed no abnormalities during physical and ultrasound examination. Concerning these findings, the urology and oncology consilium of the Medical Military Academy in Belgrade, did not suggest ipsilateral orchiectomy and the patient received three cycles of cisplatin-based chemotherapy. Two years after chemotherapy, the patient underwent left orchiectomy in another hospital. Given that histological analysis was normal, the diagnosis of the primary retroperitoneal seminoma was confirmed. The patient was followed up during the next six years. There was no relapse or metastases of the tumor.

Discussion

After the first case of extragonadal seminoma was described by Frideman [4] in 1951, there have been only a few reports of primary seminomas located in the retroperitoneum [3]. Various symptoms caused by the tumor had been described in the literature. They include enlargement of the abdomen, back pain, fever, night sweats, weight loss, venous thrombosis, testicular or groin pain, hematuria, hydrocele. Due to the slow-growing nature of the tumor and nonspecific symptoms, the seminoma has already several centimeters in size at the initial diagnosis. According to the literature, an average tumor size was around 7.2 cm [5]. However, the presented patient had much larger seminoma that has been rarely reported.

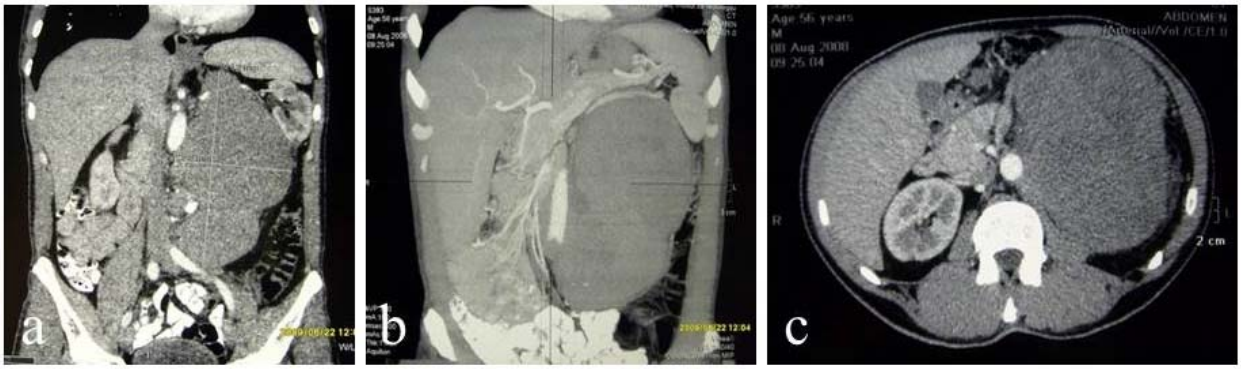


Fig. 1 – Computed tomography shows a solid nonhomogenous tumor mass in the retroperitoneum (a – frontal section), relationship with the surrounding vascular structures (b – frontal section), and the signs of aortic wall infiltration (c – axial section).

During the surgery, a firm tumor mass having 25 × 15 cm in diameter was removed from the left retroperitoneum. The small intestine, the aorta and the inferior vena cava were displaced to the right, whereas the colon descendens was pushed to the left side and partially compressed. The branches of the lumbar plexus were also compressed by the mass. The tumor adhered the *tunica adventitia* of the aorta, so that it was carefully removed from the aortic wall. This part of the aortic wall was sutured due to incomplete lesion occurred during the tumor resection. The inferior mesenteric artery was also affected and subsequently resected. The diagnosis of seminoma was made during histopathological examination. Further evaluation included biochemical analyses, lactate-dehydrogenase (LDH) and tumor markers (alphafetoprotein – AFP, beta-chorionic

Large tumor removal like in this case needs meticulous preoperative diagnostic evaluation and great attention during the surgery. The relationship between the tumor and the surrounding structures, especially with the vital organs, has to be clearly examined prior to the surgery. In this case, the seminoma was found to be adherent to the aortic wall which was not described in similar published cases. Although chemotherapy is considered the treatment of choice for huge extra-gonadal seminomas [5], we decided to perform a combination of surgery and chemotherapy due to the tumor size and severe symptoms.

In order to confirm primary seminoma in the retroperitoneum, the presence of testicular malignancies, such as metastatic testicular carcinomas, burn-out seminomas, and primary germ cell seminomas, should be excluded. Careful ultrasound

examination is suggested since it may reveal rouge pathological changes in testicles [3]. However, the findings are usually not specific for the particular tumor and carcinoma *in situ* could not be detected. Although there are eight published cases of metachronous testicular tumors that appeared up to 22 years after the removal of the retroperitoneal seminoma [2, 6], there are still no consistent suggestions whether diagnostic orchiectomy should be performed in every patient with extragonadal seminoma regardless ultrasound findings. In our case, ipsilateral orchiectomy and histopathological analysis gave the definitive diagnosis of primary retroperitoneal seminoma.

Conclusion

Although rare, the diagnosis of giant primary retroperitoneal seminoma should be considered if CT examination shows a retroperitoneal tumor mass in male patients with nonspecific symptoms. Additionally, each male patient presenting with a retroperitoneal tumor mass of unknown etiology should be examined by the urologist including evaluation of testicular tumor markers because of possible testicular tumor metastases. Surgical removal has to be performed with caution since the tumor may involve the wall of the abdominal aorta.

R E F E R E N C E S

1. *Boujelbene N, Cosinschi A, Boujelbene N, Khanfir K, Bhagwati S, Herrmann E*, et al. Pure seminoma: A review and update. Radiat Oncol 2011; 6(1): 90.
2. *Patel A, Wilson L, Rane A*. Primary retroperitoneal seminoma presenting with inferior vena caval obstruction. N Z Med J 2005; 118(1226): U1758.
3. *Chiumento C, Fiorentino A, Tartarone A, Lapadula L, Caivano R, Cozzolino M*, et al. A case report of retroperitoneal seminoma and literature review. J Nucl Med Radiat Ther 2013; 4(1): 148.
4. *Friedman NB*. The comparative morphogenesis of extragenital and gonadal teratoid tumors. Cancer 1951; 4(2): 265−76.
5. *Bokemeyer C, Droz JP, Horwich A, Gerl A, Fossa SD, Beyer J*, et al. Extragonadal seminoma. Cancer 2001; 91(7): 1394−401.
6. *Hatem MA, So B*. Primary retroperitoneal seminoma presented as gastrointestinal bleeding. J Biomed Graph Comp 2014; 4(4): 8−11.

Received on January 26, 2015.
Revised on March 20, 2015.
Accepted on March 26, 2015.
Online First April, 2015.

CASE REPORT

UDC: 617.541/.542::616.234-006-08
DOI: 10.2298/VSP141216149K

A case of endobronchial leiomyoma treated by sleeve resection of the right upper lobe bronchus

Bolesnik sa endobronhijalnim lejomiomom lečen *sleeve* resekcijom bronha za gornji režanj desnog pluća

Miloš Koledin*, Bojan Koledin*, Dejan Ilinčić*, Sladjana Koledin†

***Thoracic Surgery Department, Institute for Lung Diseases of Vojvodina, Sremska Kamenica, Serbia; †Clinic of Gynecology and Obstetrics, Clinical Center of Vojvodina, Novi Sad, Serbia**

Abstract

Introduction. Bronchial leiomyoma is extremely rare. Most reported have been resected by either lobectomy or pneumonectomy. We presented a case treated by sleeve bronchoplasty without pulmonary resection. **Case report.** The presented case, 39-year-old male, had been admitted to our hospital complaining of hemoptysis. Chest X-ray showed no abnormality in either lung field, but computed tomography scan found the tumor in the upper right bronchus. The diagnosis was made by histological and immunohistochemical examination of the specimens obtained during bronchoscopy. **Conclusion.** The presented patient was treated by thoracotomy and sleeve resection of the right upper lobe bronchus with the removal of all the tumor.

Key words:
bronchial neoplasms; leiomyoma; diagnosis; thoracic surgical procedures; treatment outcome.

Apstrakt

Uvod. Bronhijalni lejomiom je izuzetno retko oboljenje. Najčešće se takvi bolesnici leče hirurškom resekcijom, lobektomijom ili pneumonektomijom. U radu je prikazan bolesnik hirurški lečen bronhoplastičnom operacijom, *sleeve* resekcijom bronha za gornji režanj desnog pluća, bez plućne resekcije. **Prikaz bolesnika.** Muškarc, star 39 godina, primljen je u našu bolnicu zbog hemoptizija. Radiogramom njegovog grudnog koša nisu nađene nikakve patološke promene ni u jednom plućnom polju, ali je CT skenom otkriven tumor u bronhu za gornji režanj desnog plućnog krila. Dijagnoza je postavljena histološkim i imunohistohemijskim pregledom bioptata tumora, uzetog pri bronhoskopiji. **Zaključak.** Bolesnik je lečen hirurški, torakotomijom i *sleeve* resekcijom bronha za gornji režanj desnog pluća, uz otklanjanje celog tumora.

Ključne reči:
bronhusi, neoplazme; leiomiom; dijagnoza; hirurgija, torakalna, procedure; lečenje, ishod.

Introduction

Primary pulmonary leiomyomas are extremely uncommon both in adults and children, constituting approximately 2% of benign lung tumors [1]. They are thought to arise from the smooth muscle of the bronchus [2]. The affected patients usually have respiratory symptoms due to partial or complete airway obstruction which deteriorate persisting asthma [1, 2] or be complicated with bronchiectasis and recurrent pulmonary infection [1, 3, 4].

Case report

A male, 39-years old smoker, was admitted to hospital due to months' long polymorphic problems in the form of productive cough with haemoptysis, feeling of languor, fatigue and haemiparesthesia (feeling of numbness experienced on the left side of his body). Chest radiograph was normal (Figure 1), while computed tomography (CT) showed intraluminal, round, clearly circumscribed lesion in the upper lobe bronchus, 9.5 × 10 × 12.3 mm in diameter, which was post contrast homogeneously coloured without mediastinal lymphadenomegaly (Figure 2). During bronchoscopic examination, a cystic, moderately vascularized tumor change was noticed on the bronchus carina for the upper lobe bronchus, which was of smooth surface, white color, and softer consistency. The surrounding was moderately hyperemic without signs of mucosa infiltration. After bronchoscopy, a histopathological examination of the tumor change led to a leimyoma diagnosis. Histologically, tumor tissue was built of

Correspondence to: Miloš Koledin, Thoracic Surgery Department, Institute for Lung Diseases of Vojvodina, 21 234 Sremska Kamenica, Serbia. Phone: +381 64 114 39 73; Fax: +381 21 480 52 56. E-mail: drkoledin@yahoo.com

Fig. 1 Chest X-ray showed no abnormality in either lung field.

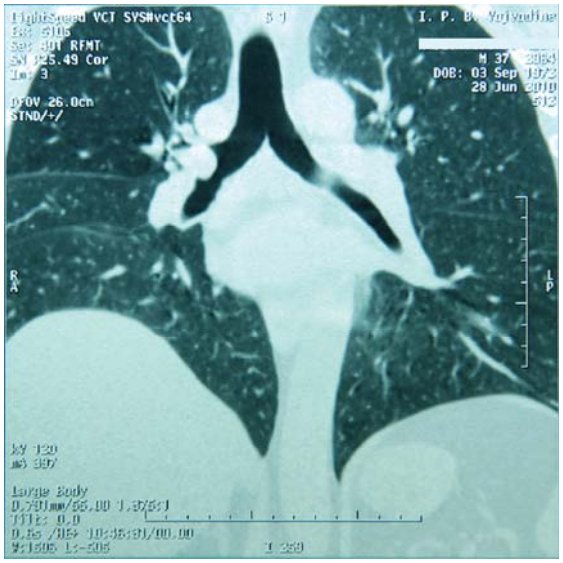


Fig. 2 − Tumor in the upper right bronchus [computed tomography (CT) scan].

intertwined bundles of spindle medium sized cells, abundant pale eosinophilic cytoplasm and elongated nuclei, yet edges that were blunt as in cigarettes. There was hyaline binder among the cells. Mitosis was not present. It was used enlargement ×10, and HE staining (Figure 3). Tumor cells were immunoreactive for desmin and alpha-SMA (Figure 4) and immunonegative for CD117, CD34, MyoD1, S-100 protein and vimentin. Due to neurological problems in the form of paraesthesia of the left part of the body, a CT examination of endocranium had been done which detected no pathological changes. After adequate preoperational preparation, the patient was operated under general anesthesia, when right anterolateral thoracotomy was performed. After cutting the initial portion of the right upper lung lobe bronchus, endoluminally was registered a smooth soft tumor, of soft consistency, whose narrow peducle went from lower lip of bronchus carina for the upper lobe bronchus towards the intermedial bronchus.

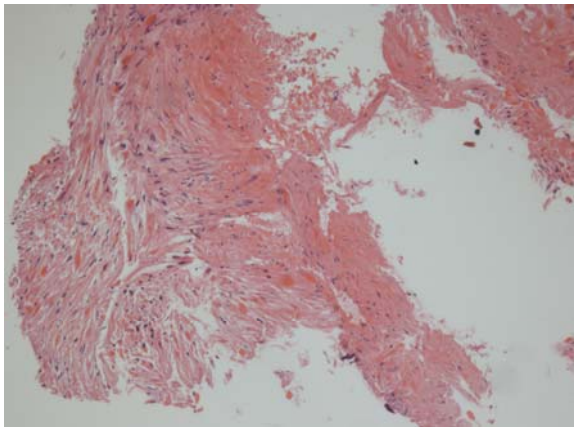


Fig. 3 − Tumor tissue [interwined bundles of spindle medium sized cells (HE, ×10)].

Circular resection of the initial part of bronchus for the upper lobe was done with tumor, wherein the section surface of the same with no tumor, as well as resectional surface of

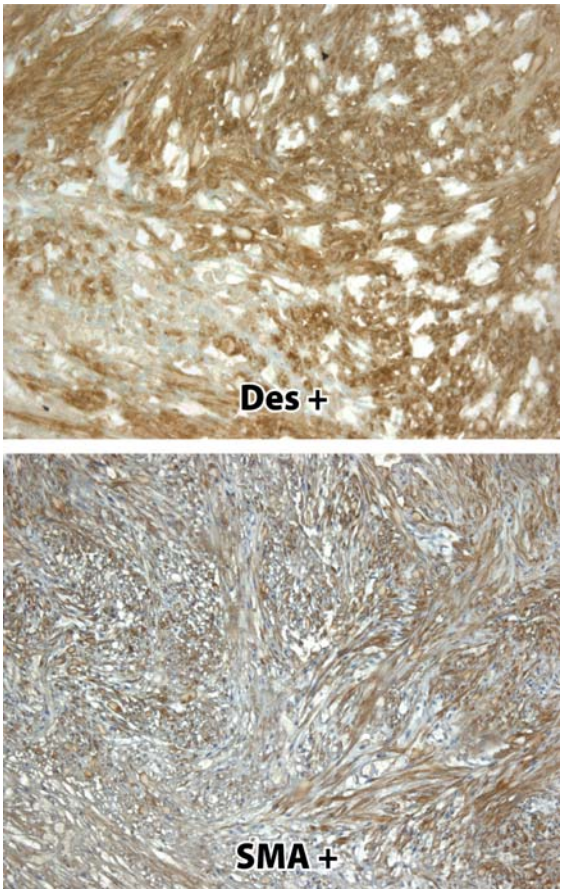


Fig. 4 − Immunohistochemical examination (×40).

the lower lip carina from intermedial bronchus were *ex tempore* histpathologically examined. After that anastomosis was performed by separate sutures. Post-operative care past with no complications. The control chest radiogram was satisfying. The control laboratory findings were within normal ranges. The definitive histopathological finding matched Hialeah leimyoma (*Leiomyoma hyalineum*).

Discussion

Leiomyomas of the lung are extremely rare and account for less than 2% of all benign tumors of the lung [1, 5, 6]. These neoplasms can occur in parenchymal, endotreacheal or endobronchial locations. Generally it seems that pulmonary leiomyomas affect females more than males with a ratio of approximately 1.5 : 1 [2, 7, 8]. Dyspnoea, cough and haemoptysis are the most common symptoms in those patients [1–4, 9]. On the chest X-ray pneumonitis is usually seen beacuse of infection, which is the result of stasis of secretion. CT scan help us to locate the tumor, but bronchoscopy is a much more helpful diagnostic procedure to define the location of the tumor, and to get histopathological diagnosis, which help us in planning the operation. When the tumor is pedunculated and small, bronchoscopic resection is also useful [10]. Bronchial leiomyomas are thought to derive from smooth muscle layer of bronchi, bronchiols, or blood vessels [11].

Conclusion

Leiomyomas of the respiratory system are essentially treated with surgical or bronchoscopic resection, depending on the location of the tumor. In some cases those tumors can be treated as conservative as possible, since the tumor is benign. In the presented case we performed a "sleeve" resection of the upper lobe bronchus, and after that anastomosis by separate sutures with the intermedial bronchus. In some other circumstances, with a secondary parenchymal destruction, leiomyomas of the bronchus may be treated with anatomic resection, like segmentectomy, lobectomy and in rare situation with pneumonectomy.

R E F E R E N C E S

1. *White SH, Ibrahim NB, Forrester-Wood CP, Jeyasingham K.* Leiomyomas of the lower respiratory tract. Thorax 1985; 40(4): 306−11.
2. *Olgun N, Ozaksoy D, Uçan ES, Türkmen MA, Canda T, Oto O,* et al. Paediatric endobronchial leiomyoma mimicking asthma. Respir Med 1995; 89(8): 581−2.
3. *Ayabe H, Tsuji H, Tagawa Y, Tomita M, Tsuda N, Chen J.* Endobronchial leiomyoma: report of a case treated by bronchoplasty and a review of the literature. Surg Today 1995; 25(12): 1057−60.
4. *Özçelik U, Kotiloğlu E, Göçmen A, Senocak ME, Kiper N.* Endobronchial leiomyoma: a case report. Thorax 1995; 50(1): 101−2.
5. *Fell CD, Tremblay A, Michaud GC, Urbanski SJ.* Electrocauterization of an Endobronchial Leiomyoma. J Bronchol 2005; 12(3): 181−3.
6. *Arrigoni MG, Woolner LB, Bernatz PE, Miller WE, Fontana RS.* Benign tumors of the lung. A ten-year surgical experience. J Thorac Cardiovasc Surg 1970; 60(4): 589−99.
7. *Perçinel S, Okçu Heper A, Savaş B, Ceyhan K, Enön S, Güngör A.* Bronchial leiomyoma with presumptive diagnosis of lung carcinoma: a case report and review of the literature. Turkish Resp J 2008; 9(1): 41−5.
8. *Vera-Román JM, Sobonya RE, Gomez-Garcia JL, Sanz-Bondia JR, Paris-Romeu F.* Leiomyoma of the lung. Literature review and case report. Cancer 1983; 52(5): 936−41.
9. *Metta H, Corti M, Redini L, Dure R, Campitelli AM, Narbaitz M.* Endobronchial leiomyoma: an unusual non-defining neoplasm in a patient with AIDS. Rev Inst Med Trop SP 2009; 51(1): 53−5.
10. *Yellin A, Rosenman Y, Lieberman Y.* Review of smooth muscle tumours of the lower respiratory tract. Br J Dis Chest 1984; 78(4): 337−51.
11. *Kim KH, Suh JS, Han WS.* Leiomyoma of the bronchus treated by endoscopic resection. Ann Thor Surg 1993; 56(5): 1164−66.

Received on December 16, 2014.
Revised on January 28, 2015.
Accepted on February 5, 2015.
Online First December, 2015.

MEETING REPORT

"Proteins and Beyond" October 12–16, 2015, Leiden University, Leiden, The Netherlands

„Budućnost proteina“ 12–16. oktobar 2015, Univerzitet u Lajdenu, Lajden, Holandija

Dragana Mučibabić

Institute for Scientific Information, Military Medical Academy, Belgrade, Serbia

Leiden University, located in the city of Leiden, is the oldest university in the Netherlands, founded in 1575 by William, Prince of Orange. The Dutch Royal Family and Leiden University still have a close relationship - Queens Juliana and Beatrix and King Willem-Alexander are all former students [1].

Leiden University includes seven faculties, over 50 departments and it is known to have an outstanding international reputation. In 2013, for example, Leiden was the highest ranked university in the Netherlands in the Times Higher Education World University Rankings, rated as the 28th best university worldwide and 61st for international reputation. Also, Shanghai Jiao Tong University's 2011 Academic Ranking of World Universities ranked Leiden University as the 29th best university worldwide. Leiden University is consistently ranked as the best university in Continental Europe for Arts and Humanities by the Times Higher Education World University Rankings. The University is associated with numerous Dutch and foreign leaders, prime ministers, a president, and even 16 recipients of the Nobel Prize (including renowned physicists Albert Einstein and Enrico Fermi). During the Dutch Golden Age (within the 17th century), scholars from around Europe were attracted to the Dutch Republic by its atmosphere of intellectual tolerance and Leiden's international reputation, thus Leiden was home to such persons as René Descartes, Rembrandt, Hugo Grotius, Baruch Spinoza and Baron d'Holbach. Now the university is a member of the Coimbra Group, the Europaeum and the League of European Research Universities [1].

Albert Einstein used to be a professor at Leiden University. Einstein regularly taught Leiden students for a few weeks per year. His first lecture at Leiden was about "Ether and Relativity Theory". It was a breaking news for scholarly circles in August 2005 that the original manuscript of a paper Albert Einstein published in 1925 had been found in the archives of Leiden University's Lorentz Institute for Theoretical Physics.

The Lorentz Institute, one of more than 40 national and international research institutes at the Leiden University was established in 1921, thus being the oldest institute for theoretical physics in The Netherlands. It is a part of the Leiden Institute of Physics together with the experimental physics groups in the Kamerlingh Onnes Laboratory and the Huygens Laboratory. The Institute Lorentz participates in two research schools, the Casimir Research School (jointly with Delft University of Technology) and the Dutch Research School of Theoretical Physics.

Ever since its foundation, the Lorentz Institute has been known for attracting scientific attention by organizing numerous events. This year, together with the Leiden Institute for Chemistry, it organized, among many others, the Workshop "Proteins and Beyond", that was held from 12 October 2015 through 16 October 2015. It brought together remarkable figures with different backgrounds and diverse methods and aspects of researching at the vast field of proteins. The most significant questions regarding the future of proteins in health and disease, the possible and desirable methodological and technical breakthroughs, including biophysical techniques for studying proteins at the molecular level, were recognized and discussed.

During the workshop numerous questions were discussed, eg molecular mechanisms of cancer development and aging, protein folding, the role of chaperons and cell membranes during folding etc. In order to better understand all of the abovementioned issues technical developments are necessary. The important observation was that in vivo protein research is becoming very powerful in visualization of proteins and therefore in studying their function in the cellular context. Still in vitro studies at the atomic level remain

Correspondence to: Dragana Mučibabić, Institute for Scientific Information, Military Medical Academy, Crnotravska 17, 11 000, Belgrade, Serbia. .: +381 11 3608 314. E-mail: vsp@vma.mod.gov.rs

essential for deeper structural understanding at the atomic level and hypothesis formulation for cellular studies.

On October 16, 2015 the three renowned speakers suggested their vision on urgent questions concerning the broad field of protein research and the technical advances expected for the coming decade.

The Chair of this public section of the workshop was Professor Claudio Luchinat (Florence University). With due respect to other scientists, especially remarkable was the appearance and the lecture of highly esteemed Professor Christopher Dobson [2] (Cambridge University):

The proliferation of amyloid disorders – A challenge for modern protein science!

After a very interesting review of great plagues ever since the era of Plato, Professor Dobson addressed the triumph of modern medicine embodied in the discovery of penicillin, and then directed our curious attention to the disease number [1] of the 20th century, especially after 1980, namely Alzheimer's disease, emphasizing it as just one out of dozens and dozens of the so-called protein-misfolding diseases.

The simple fact that amyloid disorders proliferate imposes a great challlenge on the scientists of almost any branch of research, putting the complexity and importance of proteins and their misfolding and aggregation under the special spotlight.

As having been in collusion with the proteins for millions of years, Professor Dobson explained the significance of proteins, where from they are given instructions for folding, the conceptual basis of protein folding, protein folding in a cellular context, protein misfolding as the origin of many diseases, the molecular nature of amyloid state, generic toxicity of amyloid aggregates, unified view of amyloid diseases, proteins in living organisms, the mechanisms of amyloid diseases, amyloid formation in cellular context, and many, many more quintessential aspects of this highly complex phenomena emanating from the "magic retort" of Professor Dobson.

Are we that close to resolving the mechanism(s) of amyloid disorders and, consequently, finding a proper prevention and/or remedy, remains to be seen, but the efforts to get into the heart of proteins misfolding of Professor Dobson, as well as of other scientists give us hope that it will be soon!

This highly favorable and promising atmosphere took us perfectly to the second part of this exceptional day, right into the Academy Building and the speech delivered by dear Professor Dr. Gerard W. Canters [3], the one to whom the Workshop "Proteins and Beyond" was dedicated. Professor Dr. Gerard W. Canters is widely known for his research focused on the molecular characterization of redox enzymes and proteins (mainly copper proteins) by NMR and EPR, X-ray crystallography, electrochemistry, single-molecule spectroscopy and protein engineering. It is also known that cloning of the gene for the protein azurin performed by Professor Dr. Canters in 1987 was widely accepted as new bearings in the study of structure/function relationships of blue copper proteins [3]. The extensive research of Professor Dr. Canters involves studying biological redox processes by single molecule techniques and on designing in vitro and in vivo biosensors, added to decades of scientific research and investigation and devotion to better humankind.

There have been, of course, numerous awards, as was the Royal Dutch Shell award, and each of it on this special occasion was lit up with many faces of eminent professors from all around the globe in ceremonial academic black gowns and square academic caps and the faces of all of us flocked together to greet Professor Dr. Canters with highest honors and willingly to mark together with him a new chapter of his rich scientific career.

The particular day indeed swarming with academic events and emotions was also highlighted with the other eminent participants, Prof. Thijs Aartsma, Prof. Marcellus Ubbink, at the same time highly successful organizers of the workshop, Dr. Martina Huber, to name just the three of them, but also with the PhD students, two of them heading to finishing their dissertation projects focused on protein misfolding and neurodegenerative diseases, namely Marija Mučibabić, alumna of the Department for Molecular Biology and Physiology, Belgrade University, Belgrade, Serbia and Nemanja Markešević, alumnus of the Faculty of Physics, Belgrade University, Belgrade, Serbia.

Much research has been done indeed, but scientists still have to persistently go on finding more promising techniques and analytical tools for proteins studying, combining biophysical methods, modelling cellular processes, linking in-vitro and in-cell characteristics of proteins, their misfolding tendencies under certain conditions that result in aging, and disease development. That should get scientists closer to the mechanism(s) of amyloid disorders occurrence and help in keeping proteins in their normal and functional conformation and possibly prevent or find efficient medications against those debilitating diseases that prevail over the globe in this stressful time!

R E F E R E N C E S

1. Leiden University. Available from: https://en.wikipedia.org/wiki/Leiden_University.
2. Professor Christopher Dobson. Available from: www.ch.cam.ac.uk/person/cmd44.
3. Professor Gerard W. Canters, PhD. Available from: http://www.physics.leidenuniv.nl/gwc-home.

INSTRUCTIONS TO THE AUTHORS

Vojnosanitetski pregled (VSP) publishes only papers not published before, nor submitted to any other journals, in the order determined by the Editorial Board. Any attempted plagiarism or self-plagiarism will be punished. When submitting a paper to the VSP electronic editing system, the following should be enclosed: a statement on meeting any technical requirements, a statement signed by all the authors that the paper on the whole and/or partly has not been submitted nor accepted for publication elsewhere, a statement specifying the actual contribution of each author, no conflict of interest statement that makes them responsible for meeting any requirements set. What follows subsequently is the acceptance of a paper for further editing procedure. The VSP reserves all copyrights for the published papers. Accepted are only papers in English. The VSP publishes only papers of the authors subscribed to the VSP for the year of paper submitting for publishing. Subscription is obligatory for each author/coauthor whose paper enters peer review procedure.

On January 1, 2012 the *Vojnosanitetski pregled* turned to the electronic editing system e-Ur: Electronic Journal Editing.

All the users of the system: authors, editors and reviewers have to be registered at:

http://aseestant.ceon.rs/index.php

The VSP publishes: **editorials, original articles**, **short communications**, **reviews/meta-analyses, case reports**, **medical history** (general or military), personal views, invited comments, letters to the editor, reports from scientific meetings, book reviews, and other. Original articles, short communications, meta-analyses and case reports are published with abstracts in both English and Serbian.

General review papers will be accepted by the Editorial Board only if the authors prove themselves as the experts in the fields they write on by citing not less than 5 self-citations.

Papers should be written on IBM-compatible PC, using 12 pt font, and double spacing, with at least 4 cm left margin. **Bold** and *italic* letters should be avoided as reserved for subtitles. Original articles, reviews, meta-analyses and articles from medical history should not exceed 16 pages; current topics 10; case reports 6; short communications 5; letters to the editor and comments 3, and reports on scientific meetings and book reviews 2.

All measurements should be reported in the metric system of the International System of Units (SI), and the standard internationally accepted terms (except for mm Hg and °C).

MS Word for Windows (97, 2000, XP, 2003) is recommended for word processing; other programs are to be used only exceptionally. Illustrations should be made using standard **Windows** programs, **Microsoft Office** (**Excel**, **Word Graph**)**.** The use of colors and shading in graphs should be avoided.

Papers should be prepared in accordance with the **Vancouver Convention**.

Papers are reviewed anonymously by at least two editors and/or invited reviewers. Remarks and suggestions are sent to the author for final composition. Galley proofs are sent to the corresponding author for final agreement.

Preparation of manuscript

Parts of the manuscript are: **Title page; Abstract with Key words; Text; Acknowledgements** (to the authors' desire), **References**, **Enclosures**.

1. ***Title page***

a) The title should be concise but informative, while subheadings should be avoided;

b) Full names of the authors signed as follows: ***, †, ‡, §, ||, ¶, **, ††, ...** .

c) Exact names and places of department(s) and institution(s) of affiliation where the studies were performed, city and the state for any authors, clearly marked by standard footnote signs;

d) Conclusion could be a separate chapter or the last paragraph of the discussion;

e) Data on the corresponding author.

2. ***Abstract and key words***

The second page should carry a structured abstract (250-300 words for original articles and meta-analyses) with the title of the article. In short, clear sentences the authors should write the **Background**/**Aim**, major procedures – **Methods** (choice of subjects or laboratory animals; methods for observation and analysis), the obtained findings – **Results** (concrete data and their statistical significance), and the **Conclusion**. It should emphasize new and important aspects of the study or observations. A structured abstract for case reports (up to 250 words) should contain subtitles **Introduction, Case report, Conclusion**). Below the abstract **Key words** should provide 3–10 key words or short phrases that indicate the topic of the article.

3. ***Text***

The text of the articles includes: **Introduction**, **Methods**, **Results**, and **Discussion**. Long articles may need subheadings within some sections to clarify their content.

Introduction. After the introductory notes, the aim of the article should be stated in brief (the reasons for the study or observation), only significant data from the literature, but not extensive, detailed consideratuion of the subject, nor data or conclusions from the work being reported.

Methods. The selection of study or experimental subjects (patients or experimental animals, including controls) should be clearly described. The methods, apparatus (manufacturer's name and address in parentheses), and procedures should be identified in sufficient detail to allow other workers to reproduce the results. Also, give references to established methods, including statistical methods. Identify precisely all drugs and chemicals used, with generic name(s), dose(s), and route(s) of administration. State the approval of the Ethnics Committee for the tests in humans and animals.

Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and significant aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

References

References should be superscripted and numerated consecutively in the order of their first mentioning within the text. All the authors should be listed, but if there are more than 6 authors, give the first 6 followed by *et al*. Do not use abstracts, secondary publications, oral communications, unpublished papers, official and classified documents. References to papers accepted but not yet published should be cited as "in press". Information from manuscripts not yet accepted should be cited as "unpublished data". Data from the Internet are cited with the date of citation.

Examples of references:

Jurhar-Pavlova M, Petlichkovski A, TrajkovD, Efinska-Mladenovska O, Arsov T, Strezova A, et al. Influence of the elevated ambient temperature on immunoglobulin G and immunoglobulin G subclasses in sera of Wistar rats. Vojnosanit Pregl 2003; 60(6): 657–612.

DiMaio VJ. Forensic Pathology. 2nd ed. Boca Raton: CRC Press; 2001.

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Tables

Each table should be typed double-spaced 1,5 on a separate sheet, numbered in the order of their first citation in the text in the upper right corner and supplied with a brief title each. Explanatory notes are printed under a table. Each table should be mentioned in the text. If data from another source are used, acknowledge fully.

Illustrations

Any forms of graphic enclosures are considered to bi figures and should be submitted as additional databases in the System of Assistent. Letters, numbers, and symbols should be clear and uniform, of sufficient size that when reduced for publication, each item will still be legible. Each figure should have a label on its back indicating the number of the figure, author's name, and top of the figure (**Figure 1**, **Figure 2** and so on). If a figure has been published, state the original source.

Legends for illustrations are typed on a separate page, with Arabic numbers corresponding to the illustrations. If used to identify parts of the illustrations, the symbols, arrows, numbers, or letters should be identified and explained clearly in the legend. Explain the method of staining in photomicrographs.

Abbreviations and symbols

Use only standard abbreviations. Avoid abbreviations in the title and abstracts. The full term for which an abbreviation stands should precede its first use in the text.

Detailed Instructions are available at the web site:

www.vma.mod.gov.rs/vsp

UPUTSTVO AUTORIMA

Vojnosanitetski pregled (VSP) objavljuje radove koji nisu ranije nigde objavljivani, niti predati za objavljivanje redosledom koji određuje uređivački odbor. Svaki pokušaj plagijarizma ili autoplagijarizma kažnjava se. Prilikom prijave rada u sistem elektronskog uređivanja „Vojnosanitetskog pregleda“ neophodno je priložiti izjavu da su ispunjeni svi postavljeni tehnički zahtevi uključujući i izjavu koju potpisuju svi autori da rad nije ranije ni u celini, niti delimično objavljen niti prihvaćen za štampanje u drugom časopisu. Izjavu o pojedinačnom doprinosu svakog od autora rada potpisanu od svih autora, treba skenirati i poslati uz rad kao dopunsku datoteku. Takođe, autori su obavezni da dostave i potpisanu izjavu o nepostojanju sukoba interesa čime postaju odgovorni za ispunjavanje svih postavljenih uslova. Ovome sledi odluka o prihvatanju za dalji uređivački postupak. Za objavljene radove VSP zadržava autorsko pravo. **Primaju se radovi napisani samo na engleskom jeziku.** VSP objavljuje radove samo autora koji su pretplaćeni na časopis u godini podnošenja rada za objavljivanje. Pretplata je obavezna za svakog autora/koautora čiji rad uđe u postupak recenzije.

Od 1. januara 2012. godine Vojnosanitetski pregled prešao je na e-Ur: Elektronsko uređivanje časopisa.

Svi korisnici sistema: autori, recezenti i urednici moraju biti registrovani jednoznačnom e-mail adresom. Registraciju je moguće izvršiti na:

http://aseestant.ceon.rs/index.php

U VSP-u se objavljuju **uvodnici**, **originalni članci, prethodna** ili **kratka saopštenja**, revijski radovi tipa **opšteg pregleda** (uz uslov da autori navođenjem najmanje 5 autocitata potvrde da su eksperti u oblasti o kojoj pišu), **aktuelne teme, metaanalize**, **kazuistika, seminar praktičnog lekara,** članci iz **istorije medicine**, lični stavovi, naručeni komentari, pisma uredništvu, izveštaji sa naučnih i stručnih skupova, prikazi knjiga i drugi prilozi. Radovi tipa originalnih članaka, prethodnih ili kratkih saopštenja, metaanalize i kazuistike **objavljuju se uz apstrakte na srpskom i engleskom jeziku**.

Rukopis se piše sa proredom 1,5 sa levom marginom od **4 cm**. Koristiti font veličine 12, a načelno izbegavati upotrebu **bold** i *italic* slova, koja su rezervisana za podnaslove. Originalni članci, opšti pregledi i metaanalize i članci iz istorije medicine ne smeju prelaziti 16 stranica (bez priloga); aktuelne teme – deset, seminar praktičnog lekara – osam, kazuistika – šest, prethodna saopštenja – pet, a komentari i pisma uredniku – tri, izveštaji sa skupova i prikazi knjiga – dve stranice.

U celom radu obavezno je korišćenje međunarodnog sistema mera (SI) i standardnih međunarodno prihvaćenih termina (sem mm Hg i °C).

Za obradu teksta koristiti program **Word for Windows** verzije 97, 2000, XP ili 2003. Za izradu grafičkih priloga koristiti standardne grafičke programe za **Windows**, poželjno iz programskog paketa **Microsoft Office** (**Excel, Word Graph**). Kod kompjuterske izrade grafika izbegavati upotrebu boja i senčenja pozadine.

Radovi se pripremaju u skladu sa **Vankuverskim dogovorom**.

Prispeli radovi kao anonimni podležu uređivačkoj obradi i recenziji najmanje dva urednika/recenzenta. Primedbe i sugestije urednika/recenzenata dostavljaju se autoru radi konačnog oblikovanja. Pre objave, rad se upućuje autoru određenom za korespodenciju na konačnu saglasnost.

Priprema rada

Delovi rada su: **naslovna strana, apstrakt sa ključnim rečima, tekst** rada, zahvalnost (po želji), literatura, prilozi.

1. ***Naslovna strana***

a) Poželjno je da naslov bude kratak, jasan i informativan i da odgovara sadržaju, podnaslove izbegavati.

b) Ispisuju se puna imena i prezimena autora sa oznakama redom: *, †, ‡, §, ‖, ¶, **, ††, ...

c) Navode se puni nazivi ustanove i organizacijske jedinice u kojima je rad obavljen mesta i države za svakog autora, koristeći standardne znake za fusnote.

d) Zaključak može da bude posebno poglavlje ili se iznosi u poslednjem pasusu diskusije.

e) Podaci o autoru za korespodenciju.

2. ***Apstrakt i ključne reči***

Na drugoj stranici nalazi se strukturisani apstrakt (250-300 reči za originalne članke i meta-analize) sa naslovom rada. Kratkim rečenicama na srpskom i engleskom jeziku iznosi se **Uvod/Cilj** rada, osnovne procedure – **Metode** (izbor ispitanika ili laboratorijskih životinja; metode posmatranja i analize), glavni nalazi – **Rezultati** (konkretni podaci i njihova statistička značajnost) i glavni **Zaključak**. Naglasiti nove i značajne aspekte studije ili zapažanja. Strukturisani apstrakt za kazuistiku (do 250 reči), sadrži podnaslove **Uvod, Prikaz bolesnika** i **Zaključak**). Ispod apstrakta, „Ključne reči“ sadrže 3–10 ključnih reči ili kratkih izraza koje ukazuju na sadržinu članka.

3. ***Tekst članka***

Tekst sadrži sledeća poglavlja: **uvod**, **metode**, **rezultate** i **diskusiju**. **Uvod.** Posle uvodnih napomena, navesti cilj rada. Ukratko izneti razloge za studiju ili posmatranje. Navesti samo važne podatke iz literature a ne opširna razmatranja o predmetu rada, kao ni podatke ili zaključke iz rada o kome se izveštava.

Metode. Jasno opisati izbor metoda posmatranja ili eksperimentnih metoda (ispitanici ili eksperimentne životinje, uključujući kontrolne). Identifikovati metode, aparaturu (ime i adresa proizvođača u zagradi) i proceduru, dovoljno detaljno da se drugim autorima omogući reprodukcija rezultata. Navesti podatke iz literature za uhodane metode, uključujući i statističke. Tačno identifikovati sve primenjene lekove i hemikalije, uključujući generičko ime, doze i načine davanja. Za ispitivanja na ljudima i životinjama navesti saglasnost nadležnog etičkog komiteta.

Rezultate prikazati logičkim redosledom u tekstu, tabelama i ilustracijama. U tekstu naglasiti ili sumirati samo značajna zapažanja.

U **diskusiji** naglasiti nove i značajne aspekte studije i izvedene zaključke. Posmatranja dovesti u vezu sa drugim relevantnim studijama, u načelu iz poslednje tri godine, a samo izuzetno i starijim. Povezati zaključke sa ciljevima rada, ali izbegavati nesumnjive tvrdnje i one zaključke koje podaci iz rada ne podržavaju u potpunosti.

Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa *Interneta* citiraju se uz navođenje datuma pristupa tim podacima.

Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

Balint B. From the haemotherapy to the haemomodulation. Beograd: Zavod za udžbenike i nastavna sredstva; 2001. (Serbian)

Mladenović T, Kandolf L, Mijušković ŽP. Lasers in dermatology. In: *Karadaglić Đ,* editor. Dermatology. Beograd: Vojnoizdavački zavod & Verzal Press; 2000. p. 1437–49. (Serbian)

Christensen S, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: *Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG,* editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer; 2002. p. 182-91.

Abood S. Quality improvement initiative in nursing homes: the ANA acts in an advisory role. Am J Nurs [serial on the Internet]. 2002 Jun [cited 2002 Aug 12]; 102(6): [about 3 p.]. Available from: http://www.nursingworld.org/AJN/2002/june/Wawatch.htm

Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u desnom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i simboli

Koristiti samo standardne skraćenice, izuzev u naslovu i apstraktu. Pun naziv sa skraćenicom u zagradi treba dati kod prvog pominjanja u tekstu.

Detaljno uputstvo može se dobiti u redakciji ili na sajtu: www.vma.mod.gov.rs/vsp

VOJNOSANITETSKI PREGLED
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd**, **Srbija**
Tel/Fax: +381 11 2669689
vsp@vma.mod.gov.rs

Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2016. godinu iznosi: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Pretplate: Žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 12274231295521415. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

Ime i prezime ili naziv ustanove	
Jedinstveni matični broj građana	
Poreski identifikacioni broj (PIB) za ustanove	
Mesto	
Ulica i broj	
Telefon / telefaks	

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

1. Lično. Dokaz o pretplati dostavljam uz ovu prijavu.
2. Za pripadnike MO i Vojske Srbije: Dajem saglasnost da se prilikom isplate plata u Računovodstvenom centru MO iz mojih prinadležnosti obustavlja iznos mesečne rate (pretplate).
3. Virmanom po prijemu profakture.

Datum _____	Potpis _____

VOJNOSANITETSKI PREGLED
VOJNOMEDICINSKA AKADEMIJA
Crnotravska 17, 11040 **Beograd**, **Srbija**
Tel/Fax: +381 11 2669689
vsp@vma.mod.gov.rs

Časopis „Vojnosanitetski pregled“ izlazi godišnje u 12 brojeva.

Godišnja pretplata za 2016. godinu iznosi: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za strane državljane i ustanove. Pretplate: žiro račun br. 840-314849-70 MO – Sredstva objedinjene naplate – VMA (za Vojnosanitetski pregled), poziv na broj 12274231295521415. Uplatnicu (dokaz o uplati) dostaviti lično ili poštom (pismom, faksom, *e-mail*-om). Za zaposlene u MO i Vojsci Srbije moguća je i pretplata u 12 mesečnih rata putem trajnog naloga, tj. „odbijanjem od plate“. Popunjen obrazac poslati na adresu VSP-a.

PRIJAVA ZA PRETPLATU NA ČASOPIS „VOJNOSANITETSKI PREGLED“

Ime i prezime ili naziv ustanove	
Jedinstveni matični broj građana	
Poreski identifikacioni broj (PIB) za ustanove	
Mesto	
Ulica i broj	
Telefon / telefaks	

Pretplata na časopis „Vojnosanitetski pregled“ (zaokružiti):

1. Lično. Dokaz o pretplati dostavljam uz ovu prijavu.
2. Za pripadnike MO i Vojske Srbije: Dajem saglasnost da se prilikom isplate plata u Računovodstvenom centru MO iz mojih prinadležnosti obustavlja iznos mesečne rate (pretplate).
3. Virmanom po prijemu profakture.

Datum _____	Potpis _____