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CONTENTS / SADRŽAJ

May 2014 will forever be remembered in the history of Serbia by the catastrophic floods that struck Serbia and neighboring countries, Bosnia and Herzegovina and Croatia, leaving behind numerous human casualties, demolished homes, roads, destroyed crops ... Editoral Board of the Vojnosanitetski Pregled invites all its readers and collaborators from the country and abroad to, within their capabilities, provide all necessary medical and humanitarian assistance to disaster victims.

Maj 2014. ostaće upamćen u istoriji stanovništva Srbije po katastrofalnim poplavama koje su zadesila Srbiju i susedne zemlje, Bosnu i Hercegovinu i Hrvatsku, ostavivši za sobom brojne ljudske žrtve, razrušene domove i puteve, uništenu letinu... Uredništvo „Vojnosanitetskog pregleda" poziva sve svoje čitaoce i saradnike iz zemlje i inostranstva da, u okviru svojih mogućnosti, pruže svu neophodnu medicinsku i humanitarnu pomoć postradalom stanovništvu.

SHORT COMMUNICATION

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Ejection experience in Serbian Air Force, 1990–2010

Napuštanje aviona izbacivim sedištem: analiza katapultiranja pilota Vojske Srbije u periodu od 1990. do 2010. godine

Miroslav Pavlović*†, Janko Pejović†‡, Jovan Mladenović§, Radovan Čekanac†§, Dalibor Jovanović¶, Radovan Karkalić‖, Danijela Randjelović*, Slaviša Djurdjević*

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Abstract

Background/Aim. Ejection injuries are the problem for air forces. The present risk for injuries is still too high, approximately 30–50%. The aim of this study was to determine factors responsible for and contributing to injuries in the Serbian Air Force (SAF) in the last two decades. **Methods**. All ejection cases in the SAF between 1990 and 2010 were analyzed. The collected data were: aircraft type, ejection seat generation, pilots´ age and experience, causes of ejection, aeronautical parameters, the condition of aircraft control and types of injuries. For ease of comparison the US Air Force Safety Regulations were used to define major injuries: hospitalization for 5 days or more, loss of consciousness for over 5 min, bone fracture, joint dislocation, injury to any internal organ, any third-degree burn, or second–degree burn over 5% of the body surface area. **Results.** There were 52 ejections (51 pilots and 1 mechanic) on 44 airplanes. The ejected persons were from 22 to 46 years, average 32 years. Major injuries were present in 25.49% cases. Of all the ejected pilots 9.61% had fractures of the thoracic spine, 11.53% fractures of the legs, 3.48% fractures of the arms. Of all major injuries, fractures of the thoracic spine were 38.46%. None of the pilots had experienced ejection previously. **Conclusion.** Our results suggest that taking preventive measures is obligatory. Namely, magnetic resonance imaging (MRI) scan must be included in the standard pilot selection procedure and procedure after ejection, physical conditioning of pilots has to be improved, training on ejection trainer has to be accomplished, too.

Key words:
aerospace medicine; military personnel; occupational exposure; accidents aviation; wounds and injuries; serbia.

Apstrakt

Uvod/Cilj. Povrede nastale katapultiranjem predstavljaju problem za ratno vazduhoplovstvo. Rizik od nastajanja povreda još uvek je visok i kreće se od 30% do 50%. Cilj ove studije bio je da se odrede faktori koji doprinose povredama u vazduhoplovstvu (V) i protivvazdušnoj odbrani (PVO) Vojske Srbije u poslednje dve dekade. **Metode.** Analizirani su svi slučajevi katapultiranja u V i PVO Vojske Srbije u periodu 1990–2010. Prikupljeni podaci odnosili su se na: tip vazduhoplova, generaciju (tip) izbacivog sedišta, starost pilota, iskustvo sa katapultiranjem, uzrok katapultiranja, aerodinamičke parametre koji prethode katapultiranju (vazdušna brzina, visina, položaj vazduhoplova), stanje upravljivosti aviona, vreme iskakanja, težina povreda (teške telesne povrede – TTP; lake telesne povrede – LTP; bez povreda). Zbog mogućnosti lakšeg poređenja sa drugim zemljama, korišćena je klasifikacija Američkog ratnog vazduhoplovstva za teške telesne povrede koja podrazumeva: bolničko lečenje preko pet dana, gubitak svesti preko 5 minuta, prelome kostiju, iščašenje zglobova, povrede unutrašnjih organa, sve opekotine III stepena, sve opekotine II stepena koje zahvataju preko 5% površine tela. **Rezultati.** U navedenom periodu bilo je 52 katapultiranja (51 pilot i jedan mehaničar letač), na ukupno 44 aviona. Starost pilota bila je u rasponu od 22 do 46 godina, prosečno 32 godine. Teške telesne povrede bile su zastupljene kod 25,49% pilota. Od svih katapultiranih pilota 9,61% imalo je prelome torakalne kičme, 11,53% prelome nogu, 3,48% prelome ruku. Od svih TTP prelom torakalne kičme bio je zastupljen kod 38,46% katapultiranih pilota. Niko od pilota nije imao prethodno iskustvo sa katapultiranjem. **Zaključak.** Naši rezultati ukazuju da je neophodno sprovođenje mera prevencije. Magnetna rezonanca mora biti uključena u standardnu proceduru selekcije pilota, kao i u proceduru nakon katapultiranja. Potrebno je podići nivo fizičke kondicije. Takođe, potrebno je vršiti obuku na trenažeru izbacivog sedišta.

Ključne reči:
medicina, vazduhoplovna; kadar, vojni; profesionalna izloženost; udesi, vazduhoplovni; rane i povrede; srbija.

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Introduction

Emergency escape from aircraft has been of utmost importance to air force since its inception. Safety and survival of crewmembers have been a major thrust of the entire safety program.

Although survival rates, nature of injuries, and reasons for ejection have been investigated for various air forces and show different characteristics, ejection injuries are still the problem for air forces.

The present risk of injuries is too high, approximately 30–50%. The aim of this study was to determine factors responsible for and contributing to injuries in the Serbian Air Force (SAF) in the last two decades.

Fig. 1 – The plain type J-22 (Orao in Serbian)

Methods

All ejection cases in the SAF between 1990 and 2010 were analyzed. The collected data were: type of aircraft, generation of ejection seat, pilots´ age, pilots´ experience, causes of ejection, aeronautical parameters, the condition of aircraft control types of injuries (major, minor, non-injury). For ease of comparison, the US Air Force Safety Regulations were used to define major injuries: hospitalization for 5 days or more, loss of consciousness for over 5 min, fracture of bone, dislocation of joint, injury to any internal organ, any third-degree burn, or second–degree burn over 5% of body surface area.

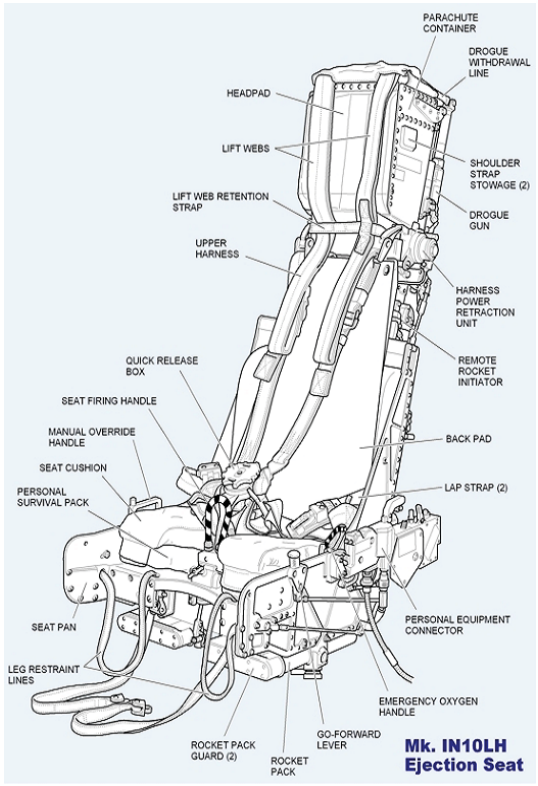

Fig. 2 – The ejection seat – Martin Baker 10

Results

There were 52 ejections (51 pilots and 1 mechanic) on 44 airplanes. The ejected pilots were 22 to 46 years old, average 32 years. The pilots with major injuries had 32.8 years on the average. Emergencies that required ejection were: engine failure (3), control system failure (6), gear failure (5) mid-air collision (1) bird collision (2), and war action (23).

Seven different types of planes were used with five types different-generation ejection seat. Plane and ejection seat types with the major injuries were J/22 (Figure 1) and Martin Baker (MK/Y10) (Figure 2), respectively.

Involvement of Martin-Baker (MK-10) ejection seat in relation to all ejection seats was 53.8%, and involvement of MK-10 in the major injuries was 69.2%.

Involvement of KM-1 seat in a total number of ejections was 13.46%, and in major injuries 22.22% (Table 1).

Table 1

Aircraft, ejection seat and injuries types in the Serbian Air Force ejection expiriences during 1990–2010

Plane type	Pilots (n)	Ejection seat type	Major injuries (n)	Minor injuries (n)	No injuries (n)
H- 62 (9)	11	Martin-Baker (MK-YU 10)	2	3	6
J - 22, HJ-22 (12)	17	MK-YU 10	7	2	8
JI-17 (7)	7	KM-1	3	1	3
J – 21 (7)	7	Foland 1B	1	2	4
JI –14 (2)	2	KM			2
JI –18 (5)	5	K–36 ДМ		1	4
H– 60 (2)	2	Foland 1B			2
Total: 44	51		13 (25.49%)	9 (17.64%)	29 (56.86%)

It is obvious that major injuries were present in 25.49% cases suggesting that every fourth pilot had experienced major injury. Major injuries in war action were only 2, namely15.3% of all major injuries. It should be noted that there was no major injuries with K-36ДМ, in spite of ejections in war actions.

A list of injuries included: *fractura fibulae l.sin*.; *frac. mal. lat. cruris. sin*.; *fractura oss nasale*; *fractura* Th – IV; *frac. subcapitis ossis methacarp*. II *manus dex. aperta*; *frac*. Th – VIII; *frac. tibiae*; *frac. costae* X l. *sin; frac*. *compresiva* Th – VIII; frac. Th - X – XII, *cum fractura cruris sin. gr*. I *aperta*; *frac*.Th - IX , *spondilodesis* Th -VII –XI.

Of all the ejected pilots, 5 (9.61%) had fractures of the thoracic spine, 6 (11.53%) fractures of the legs, and 2 (3.48%) fractures of the arms. Two of them had united fractures.

Of all the major injuries, 38.46%, were related to fractures of thoracic spine, and 60% of them were inflicted on the plain J-22 (MK-10).

Minor injuries that should be mentioned were laceration of the face and burns of the arms.

Obesity (adipositas) was presented in 23.07% of the pilots with major injuries.

None of the pilots had previously experienced ejection.

Discussion

Analysis done by foreign air forces for long periods of time, with different types of planes and generations of seats, revealed different degrees of fatal injuries during ejection. The highest degree of fatal injuries was recorded in the Japanese Air Force, 22.9% of mortal outcomes in a study for a period 1956–2004 [1]. The main reason was the delay in making decision for ejection.

In the study on accidents from 1973 to 1985 US Air Force (USAF) presented a survival rate of 86% [2]. Swedes, in their study for a period 1967–1987 claimed 83 successful ejections and 9 fatal outcomes [3]. Finns, in the study from 1958 to 1991 quoted survival rate higher than 80% [4]. English, in the study of 232 cases of ejection, for a period 1973–2002, quoted the survival rate of 89.2% [5]. In our study there was no case of ejection with fatal outcome.

Compression a fracture of the spine is a common consequence of ejection. Finns quoted 18% of such cases in the total number of all major injuries, Swedes 25%, Italians 15%, USAF 6%, Japanese 63% and English 29.4% of all aircrew. Germans quoted 17.6% of spine fractures in their Air Force, for a period from 1981 to 1997 [6]. Americans quoted 6 spine fractures from 18 ejections in the "Desert Storm" [6].

In our survey spine fractures were presented in 9.61% of all ejections. The incidence of spine injures was 38.46% of the major injuries and 60% of all spine fractures was on plane J-22(MK-10).

In their study, English quoted that 44% of minor spinal compression fractures and injuries of spinal ligaments could not be diagnosed with classic Roentgen recording, but only with magnetic resonance [5]. This emphasizes the importance of examination with magnetic resonance of all aircrews after ejections.

Irregular seating position during ejection was accused to be the main reason for spinal fractures and a combination of accomplished highest acceleration and rate of onset. The injuries appeared in the moment of discharge, and acceleration upward. It was established that every reduction of acceleration in the moment of discharge reduces forces acting on the spine and the degree of spine injures. It was concluded that acceleration reduction from 24 m/s to 18 m/s reduces rate of injuries [5]. The highest rate of injuries was on a plane Tornado, with Martin-Baker seats Mk-10A, with the speed of 20.7 m/s, compared with 19.5 m/s for other types of planes [5].

In our case, the estimated speed for MK-YU10(MK-10) was 19.8 m/s.

It should be pointed out that a connection between spine fractures and anthropometric measures of pilots could not be established [5].

In war action the rate of major injuries during ejection was lower than it could be expected. A possible reason was the participation of most experienced aircrews.

In their study Swedes quoted that two third of successfully ejected pilots returned to job after 1 week, others were absent for one year, and only 3.5% finished their flying career [3].

Conclusion

Risk of injuries during ejection still remains too high, approximately 30–50%, in our survey 25.49%. There were no ejections with fatal outcome in our study. The main reason for spine injuries was irregular position of the spine in the seat and a combination of the peak of acceleration and the rate of onset. Preventive measures must be promoted: MRI scan should be included in the standard selection procedure and procedure after ejection physical conditioning has to be improved, training on ejection trainer has to be accomplished, too.

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A cephalometric analysis of the cranial base and frontal part of the face in patients with mandibular prognathism

Kefalometrijska analiza kranijalne baze i prednjeg dela lica kod osoba sa mandibularnim prognatizmom

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Abstract

Bacground/Aim. The literature suggests different views on the correlation between the cranial base morphology and size and saggital intermaxillary relationships. The aim of this study was to investigate the cranial base morphology, including the frontal facial part in patients with mandibular prognathism, to clarify a certain ambiguities, in opposing viewspoints in the literature. **Methods.** Cephalometric radiographies of 60 patients were analyzed at the Dental Clinic of the Military Medical Academy, Belgrade, Serbia. All the patients were male, aged 18–35 years, with no previous orthodontic treatment. On the basis of dental and sceletal relations of jaws and teeth, the patients were divided into two groups: the group P (patients with mandibular prognathism) and the group E (the control group or eugnathic patients). A total of 15 cephalometric parametres related to the cranial base, frontal part of the face and sagittal intermaxillary relationships were measured and analyzed. **Results.** The results show that cranial base dimensions and the angle do not play a significant role in the development of mandibular prognathism. Interrelationship analysis indicated a statistically significant negative correlation between the cranial base angle (NSAr) and the angles of maxillary (SNA) and mandibular (SNB) prognathism, as well as a positive correlation between the angle of inclination of the ramus to the cranial base (GoArNS) and the angle of sagittal intermaxillary relationships (ANB). *Sella turcica* dimensions, its width and depth, as well as the nasal bone length were significantly increased in the patients with mandibular prognathism, while the other analyzed frontal part dimensions of the face were not changed by the malocclusion in comparison with the eugnathic patients. **Conclusion**. This study shows that the impact of the cranial base and the frontal part of the face on the development of profile in patients with mandibular prognathism is much smaller, but certainly more complex, so that morphogenetic tests of the maxillomandibular complex should be included in further assessment of this impact.

Key words:
mandible; prognathism; cephalometry; skull; facial bones; sella turcica.

Apstrakt

Uvod/Cij. U litetaruri postoje različiti stavovi o povezanosti morfologije i veličine kranijalne baze i sagitalnih međuviličnih odnosa. Cilj ovog rada bio je da se ispita morfologija kranijalne baze, uključujući i prednji deo lica, kod ispitanika sa mandibularnim prognatizmom da bi se razjasnile nedoumice donekle suprotnih stavova u literaturi. **Metode.** Analizirani su rendgenkefalometrijski snimci ukupno 60 bolesnika Klinike za stomatologiju VMA. Svi bolesnici bili su muškog pola, starosti od 18 do 35 godina koji ranije nisu bili ortodontski lečeni. Bolesnici su prema dentoskeletnim odnosima vilica i zuba bili svrstani u dve grupe: grupu P (bolesnici sa mandibularnim prognatizmom) i grupu E (kontrolna grupa ili grupa eugnatih bolesnika). Izmereno je i analizirano 15 kefalometrijskih parametara koji su se odnosili na kranijalnu bazu, frontalni deo lica kao i sagitalne međuviličnе odnose. **Rezultati.** Dobijeni rezultati ukazuju da ni dimenzije kranijalne baze, ni njen ugao ne igraju značajnu ulogu u nastanku mandibularnog prognatizma. Analizom me-

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đuzavisnosti ustanovljeno je da postoji statistički značajna negativna korelacija između ugla kranijalne baze (NSAr) i uglova maksilarnog (SNA) i mandibularnog (SNB) prognatizma, kao i pozitivna korelacija između ugla nagiba ramusa prema kranijalnoj bazi (GoArNS) i ugla sagitalnih međuviličnih odnosa (ANB). Dimenzije sedlaste jamice (*sella turcica*), njena širina i dubina, kao i dužina nosne kosti statistički su značajno povećane kod bolesnika sa mandibularnim prognatizmom, dok ostale analizirane dimenzije prednjeg dela lica nisu bile izmenjene kod ove malokluzije u odnosu na eugnate bolesnike. **Zaključak**. Pokazalo se da je uticaj kranijalne baze i prednjeg dela lica na ispoljavanje profila kod bolesnika sa mandibularnim prognatizmom mnogo manji ali svakako složeniji, pa bi u dalja istraživanja trebalo uključiti morfogenetska ispitivanja maksilomandibularnog kompleksa kod ocenjivanja ovog uticaja.

Ključne reči:
mandibula; prognatizam; kefalometrija; lobanja; lice, kosti; sela turcika.

Introduction

The cranial base plays an important role in the development of face, especially in achieving sagittal and vertical intermaxillary relationships, primarily because of the different ways of ossification of its synchondrosis. It also represents a central skeletal axis which achieves its final size very early – long before the face.

All the bones that form the cranial base (apart from temporal) are of cartilaginous origin and created by enchodral ossification which already begins prenatally and ends in early childhood (especially the growth of sphenoethmoidal and sphenofrontal synchondroses ends early), following the growth of sphenooccipital synchondroses which is completed approximately at the age of 12–16, so that the length of the frontal cranial base becomes defined in a very early period [1, 2]. In postnatal period, especially in puberty, the frontal sinus enlargement and remodelling of its frontal surface occur, which also influence the nasal bone [3].

The opinions, that the growth, dimensions and shape of the cranial base influence the middle face growth have been accepted. Apposition and remodelling of the cranial base sutures until the age of 5 affect the growth and position of the maxilla, thus forming the maxillary sagittal position to the cranial base very early. Afterwards, when the growth of the cranial base sutures stops, it is replaced by the growth of the sutures connecting the maxilla with the cranial base, thus moving the maxilla forward and downwards. According to another theory, the growth of the entire cranial complex gradually decreases from the age of 3–7 years, when remains active only in the mandibular condyle, so that the mandible grows smoothly, changing partially its sagittal position to the cranial base until general somatic growth is completed [4].

Anatomically speaking, the middle face is set in such a way that the maxilla is attached to the anterior cranial base by its sutures, whereas the mandible is connected to the posterior cranial base by the temporomandibular joint. Due to the anatomy, any change in dimensions or the angle of the cranial base, results in changes of the maxilla or mandible position as well as their interrelationship.

Many cephalometric studies have confirmed that the shape and dimensions of the cranial base vary in patients with different sagittal intermaxillary relationships [4–10]. Mandibular prognathism (MP) is a genetic, complex craniodento-facial developmental disorder, where disordered intermaxillary sagittal and vertical relationships dominate, primarely as a result of overdevelopment of the mandible. There are still dilemmas, whether the cranial base really plays such a decisive role in etiology, as the authors have often reported. In mandibular prognathism, the cranial base angle is sharper and the cranial base is shorter in comparison with skeletal Class I patients, while the case of skeletal Class II patients is completely opposite [3, 5, 6, 10].

Some authors believe that the temporomandibular joint is placed in more anterior position precisely because of the reduction of the cranial base angle, which results in a prognathic facial profile. However, Singh et al. [6, 7, 9] and Proff et al. [5] have demonstrated in their extensive studies that the biological basis of anterior positioning of the temporomandibular joint lies in the posterior cranial base. The same authors suggest that the reason could be the premature cessation of the growth of petro-spheno-occipital complex, in other words, that a premature synostosis is responsible for deficient orthocephalization (horizontalization) of the cranial base angle in Class III malocclusion. Therefore, the reduced posterior part of the cranial base can be a primary factor in skeletal Class III etiology.

Consequently, the shape of cranial base could determine facial profiles and represent the key factor in developing skeletal class malocclusions. Is this really true since that MP is a developmental disorder, which reaches its full manifestation until after puberty, and the cranial base ossification occurs in early childhood?

In almost all cephalometric analyses of the neurocranium and the viscerocranium, *sella turcica* (ST) takes a central place. More precisely, the cental point (*sella* point – S) is a part of many reference planes by which other structures are oriented. Thus, ST shape, dimensions and position in relation to the surronding structures are of great importance. For a long time, authors have had a tendency to determine ST dimensions as precise as possible, primarily because of its close relationship with a pituitary gland. Today, however, it is well-known that ST enlargement does not imply that the pituitary gland is also enhanced, and *vice versa* [1, 11–13].

It has been found that changes in ST shape and dimensions are caused by many congenital anomalities: cleft lip and palate [14], lumbosacral myelomeningocele [15], Seckel syndrome [16], Rieger's syndrome [17], congenital craniofacial deviations [18–20], even by congenital dental anomalies, such as a palatal position of the upper fangs and hypodontia of mandibular second premolars [21]. A *sella turcica* bridge in pa-

tients with various craniofacial deviations treated by surgical-orthodontic means to correct the existing deformities was investigated by Becktor et al. [18], Jones et al. [19] and Alkofide [22]. All the authors found significant differences between study groups and general population, emphasizing that the majority of patients with craniofacial deviations were later treated by surgery on mandible.

Čutović et al. [20], analyzing *sella turcica* dimensions in patients with mandibular prognathism, found that all the three ST measured dimensions (surface, width and depth) were significantly higher in patients with mandibular prognathism than in eugnathic subjects, but the degree of the manifested anomaly did not have any influence on the size of changes in the abovementioned dimensions.

The anterior cranial base, whose growth ends very early, has a weak influence on positioning the frontal facial parts, that is, only the orbital part directly depends on it. Since the floor of eye socket is also the roof of maxilla, Enlow [4] assumed that the dimensions and the position of the orbital cavity should be correlated with the position of maxilla. However, Holly et al. [23] tested this hypothesis on 32 primates and found that the correlation was too weak.

The growth of the frontal facial parts later in puberty is mostly seen in the increase of the frontal sinuses volume changing the shape of supraorbital ridge and also indirectly affecting the nasal bone [3]. Singh et al. [9] found that the elongation of the anterior cranial base, particularly around the age of 9, directly influenced the enlargement of the frontal sinus, significantly affecting the morphological changes of supraorbital and nasal structures. In the literature available to us, we found that the cephalometric changes of supraorbital ridge and frontal sinus had been recorded only by Dostalova et al. [24], who investigated a number of cephalometric abnormalities in patients with acromegaly, and among other things, came to a conclusion that the frontal sinus was increased and the supraorbital ridge pronounced in these patients in comparison with the control group. The changes were more prominent in men than women and did not depend on the growth hormone, but on the duration of the illness.

The nasal bone consists of two bones, forming the skeleton of nose and is located between the frontal extensions of the maxilla and frontal bone.

Dostálová et al. [24] measured the nasal bone length and inclination to the cranial base in healthy subjects and patients with acromegaly and found that the dimensions of nasal bone were not changed in patients with acromegaly.

Singh et al. [9] found a negative correlation between the frontonasal angle and the cranial base angle. In patients with skeletal Class III, the cranial base angle is normally reduced, so that the frontonasal angle is increased, resulting in a flat midface profile, which is a common feature of this dentofacial deformity.

The aim of this study was to conduct a cephalometric analysis of morphological characteristics of the cranial base, including *sella turcica* and frontal facial part (supraorbital ridge, frontal sinus, nasal bone) in patients with mandibular prognathism, as well as of their correlation with the indicators of sagittal intermaxillary relationships.

Methods

Lateral cephalometric images of 60 orthodontic patients, were taken and analyzed before their treatment at the Dental Clinic, Military Medical Academy.

Using the findings from the literature on gender differences and growth changes dynamics [25, 26], we decided to study male subjects, aged 18–30 years.

The group P consisted of 30 patients with mandibular prognathism, diagnosed on the basis of the following criteria: the angle of mandibular prognathism (SNB) $\geq 80°$; the angle of sagittal intermaxillary relationship (ANB) $\leq 0°$; the angle B $\geq 30°$; Bjork $\geq 396°$; reverse overlap of the frontal teeth and relationship of the first permanent molars in Class III.

The control group, the group E, consisted of 30 patients with normal intermaxillary relationships (skeletal Class I, eugnathic subjects): SNB $\leq$ 80°; ANB = 0 - 5°; normal overlap of the frontal teeth and relationship of the first permanent molars in Class I.

All the patients from the group P were planned for and later treated with orthodontic-surgical therapy, which was performed by the same team.

A cephalometric analysis

Lateral cephalometric images of the head were taken for each patient under standard conditions. The head was fixed in a cephalostat, and recording conducted at the distance of 1.5 m. Analysis of lateral cephalogram images was preceded by drawing the corresponding structures on a tracing paper fixed on a film. Afterwards, numerous points and planes were marked for analyzing certain angular and linear parameters taken from the analyses of Steiner, Jacobson, Ricketts, Downs and Bjork. Measurements were performed twice by the same examiner, on different days, with the accuracy of 0.5 mm or 0.5°. Statistically significant differences did not appear between these two measurements.

Analysis of the following cephalometric parameters was carried out between the patients with mandibular prognathism and the control group of eugnathic patients: SN – the anterior cranial base length; SAr – the posterior cranial base length; NAr – the total cranial base length; NSAr – the cranial base angle; SG – supraorbital ridge; F1F2 – the frontal sinus range; SGN – the angle of protrusion of the supraorbital ridge; Ss – the width of *sella turcica* (the largest anteroposterior diameter); Ds – the depth of *sella turcica* (from the line connecting clinoid extensions to the lowest point of the floor); NR – the nasal bone length; SNR – the angle of the inclination of the nasal bone; SNA – the angle of maxillary prognathism; SNB – the angle of mandibular prognathism; ANB – the angle of sagittal intermaxillary relationships; GoArNS – the angle of inclination of the ramus to the cranial base (Figure 1).

According to the data collected by lateral cephalometric analysis for each patient and each feature, the database was formed in the SPSS12 Program for Windows and the following statistical methods were used in the statistical analysis: tables and graphical presentations, descriptive statistics methods, the Bonferroni test for detecting intergroup differences and the linear correlation method.

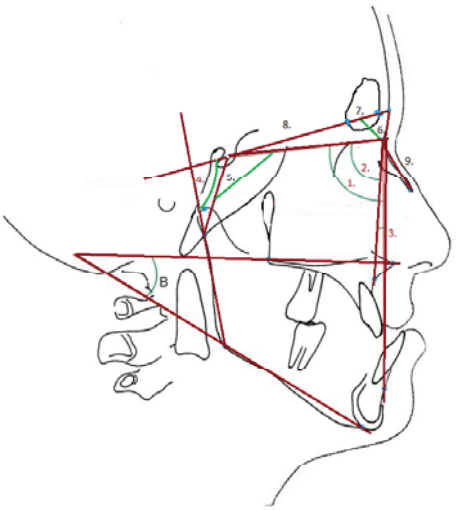

Fig. 1 – Angular and linear measurements:
1. SNA – maxillary prognathism angle; 2. SNB – mandibular prognathism angle; 3. ANB – angle of sagittal intermaxillary relationships; 4. GoArNS – angle of inclination of the ramus to the cranial base; 5. NSAr – cranial base angle; 6. SGN – protrusion angle of the supraorbiatal ridge; 7. F1F2 –frontal sinus range; 8. SG – supraorbital ridge; 9. NR – nasal bone length.

Results

Tables 1, 2 and 3 show the statistical results of analyzing the following parameters of the cranial base, the frontal facial part and sagittal intermaxillary relationships: the anterior cranial base (SN), the posterior cranial base length (SAr), the total cranial base length (NAr), the cranial base angle (NSAr), supraorbital ridge (SG), the frontal sinus range (F1F2), the angle of protrusion of the supraorbital ridge (SGN), the width of *sella turcica* (Ss), the depth of *sella turcica* (DS), the nasal bone length (NR), the angle of inclination of the nasal bone (SNR), the angle of maxillary prognathism (SNA), the angle of mandibular prognathism (SNB), the angle of the sagittal intermaxillary relationships (ANB), the angle of inclination of the ramus to the cranial base (GoArNS).

Sella turcica (Ss) width showed higher values in the patients with mandibular prognathism than in the eugnathic subjects. The average value of the *sella turcica* width in the eugnathic subjects is 9.53 mm ± 1.34, whereas it was 11.07

Table 1

Analyzed parameters values for the cranial base in the eugnathic subjects (E) and the patients with mandibular prognathism (P) (descriptive statistical indicators)

Analyzed parameters		n	x̄	SD	Min	Max
S-N	E	30	77.90	4.20	65.00	84.50
	P	30	76.07	4.07	69.00	83.00
	Total	60	77.40	4.20	65.00	88.00
S-Ar	E	30	39.30	3.82	31.00	47.00
	P	30	37.37	3.62	29.00	45.50
	Total	60	38.51	3.94	29.00	47.00
NAr	E	30	104.08	7.03	88.00	117.00
	P	30	100.13	6.79	90.00	116.00
	Total	60	101.66	11.57	11.50	117.00
NSAr	E	30	120.55	5.59	110.00	133.00
	P	30	118.90	7.81	101.00	135.00
	Total	60	119.32	6.58	101.00	135.00
Ss	E	30	9.53	1.34	6.50	12.00
	P	30	11.07	1.45	7.50	15.00
	Total	60	10.49	1.61	6.00	15.00
Ds	E	30	7.55	1.75	3.00	11.00
	P	30	9.33	1.66	6.00	13.00
	Total	60	8.38	1.73	3.00	13.00

SN – anterior cranial base length; SAr – posterior cranial base length; NAr – total cranial base length; NSAr – cranial base angle; Ss – width of *sella turcica* (the largest anteroposterior diameter); Ds – depth of *sella turcica* (from the line connecting clinoid extensions to the lowest point of the floor).

Table 2

Analyzed values parameters of the frontal facial part in the eugnathic subjects (E) and patients with mandibular prognathism (P) (descriptive statistical indicators)

Analyzed parameters		n	x̄	SD	Min	Max
F1-F2	E	30	14.43	4.50	5.00	25.00
	P	30	14.07	2.69	8.00	19.00
	Total	60	14.53	3.86	5.00	25.00
S-G	E	30	84.27	4.48	71.00	91.50
	P	30	82.85	4.89	74.00	90.00
	Total	60	84.26	4.58	71.00	96.00
SGN	E	30	57.20	5.49	38.50	66.00
	P	30	54.73	6.49	41.00	66.00
	Total	60	55.95	5.90	38.50	72.00
NR	E	30	24.70	3.54	17.00	33.00
	P	30	27.58	3.65	20.00	35.00
	Total	60	26.40	3.57	17.00	35.00
SNR	E	30	118.10	9.34	104.00	135.00
	P	30	117.82	6.92	105.00	133.00
	Total	60	118.98	7.40	104.00	135.00

F1F2 – frontal sinus range; SG – supraorbital ridge; SGN – angle of protrusion of the supraorbital ridge; NR – nasal bone length; SNR – inclination angle of the nasal bone.

Table 3

Analyzed parameters values of the sagittal intermaxillary relationships in the eugnathic subjects (E) and the patients with mandibular prognathism (P)

Analyzed parameters		n	x̄	SD	Min	Max
SNA	E	30	82.38	4.05	73.00	89.00
	P	30	77.67	4.29	71.00	86.50
	Total	60	79.94	4.28	71.00	89.00
SNB	E	30	79.30	4.18	72.00	87.50
	P	30	83.92	2.74	77.00	90.50
	Total	60	83.54	4.73	72.00	93.00
ANB	E	30	3.15	1.70	0.50	7.00
	P	30	-6.22	3.13	-12.00	-0.50
	Total	60	-3.54	5.50	-15.00	7.00
GoArNS	E	30	82.58	5.34	71.00	94.00
	P	30	80.18	5.13	72.00	99.00
	Total	60	79.73	5.65	68.00	99.00

SNA – angle of maxillary prognathism; SNB – angle of mandibular prognathism; ANB – angle of sagittal intermaxillary relationships; GoArNS – angle of inclination of the ramus to the cranial base.

mm ± 1.45 in the patients with mandibular prognathism. Table 4 shows a statistically significant difference in the Ss values between the group E and the group P ($p < 0.001$).

Sella turcica depth (Ds) shows higher values in the patients with mandibular prognathism than in the eugnathic subjects. The average value of the depth of *sella turcica* in the eugnathic subjects is 7.55 mm ± 1.75, whereas it is 9.33 mm ± 1.66 in the patients with mandibular prognathism. Table 4 shows a statistically significant difference in the Ds values between the group E and the group P ($p < 0.001$).

Nasal bone length (NR) showed higher values in the patients with mandibular prognathism than in the eugnathic subjects. The average value of the nasal bone length in the eugnathic subjects is 24.70 mm ± 3.54, whereas it was 27.58 mm ± 3.65 in the patients with mandibular prognathism. Table 4 shows a statistically significant difference in the NR values between the group E and the group P ($p < 0.001$).

Maxillary prognathism angle (SNA) shows higher values in the eugnathic subjects than in the patients with mandibular prognathism. The average value of the SNA in the eugnathic subjects is 82.38 ± 4.05, whereas it is 77.67 ± 4.29 in the patients with mandibular prognathism. Table 4 shows a statistically significant difference in the SNA values between the group E and the group P ($p < 0.001$).

Mandibular prognathism angle (SNB) shows higher values in the patients with mandibular progmathism than in the eugnathic subjects. The average value of the SNB in the eugnathic subjects is 79.30 ± 4.18, whereas it was 83.92 ± 2.74 in the patients with mandibular prognathism. Table 4 shows a statistically significant difference in the SNB values between the group E and the group P ($p < 0.001$).

Sagittal intermaxillary relationships angle (ANB) shows higher values in the eugnathic subjects than in the patients with mandibular prognathism. The average value of this angle in the eugnathic subjects is 3.15 ± 1.70, whereas it is -6.22 ± 3.13 in the patients with mandibular prognathism. Table 4 shows a statistically significant difference in the ANB values between the group E and the group P ($p < 0.001$).

The angle of ramus inclination to the cranial base (GoArNS) shows higher values in the eugnathic subjects than in the patients with mandibular prognathism, but they are statistically insignificant**.** The average value of this angle in the eugnathic subjects is 82.58 ± 5.34, whereas it is 80.18 ± 5.13 in the patients with mandibular prognathism.

For the remaining cranial base parameters (SN, SAr, NAr, NSAr, SG, F1F2, SGN, SNR), the Bonferroni test did not show any statistically significant difference between the two groups of examinees (Table 4).

Table 4

Boniferri test results, examining intergroup differences (eugnatics subjects *vs* patients with mandibular prognatism) by using all the cranial base, frontal facial part and sagittal intermaxillary relationships analyzed parameters

Parameters	Differences in average values	*p*
Ss	-1.53333	0.000
Ds	-1.78333	0.000
NR	-2.88333	0.004
SNA	4.72	0.000
SNB	-4.62	0.000
ANB	9.37	0.000

Ss – *sella turcica* width; Ds – *sella turcica* depth; NR – nasal bone length; SNA – angle of maxillary prognathism; SNB – angle of mandibular prognathism; ANB – the angle of sagittal intermaxillary relationships.

By analyzing interrelationships between 5 parameters in mandibular prognathism: (NSAr, SNA, SNB, ANB, and GoArNS, given in Table 5, a statistically significant and highly negative correlation was found between the cranial base angle and NSAr and SNA ($p = -0.567$) and SNB ($p = -0.676$) angles. The GoArNS showed a statistically significant

and positive correlation with the ANB ($p = 0.385$), whereas the SNA and SNB angles showed a statistically significant, interrelated and positive correlation ($p = 0.674$) (Table 5).

Table 5
Correlation matrix of the analyzed parameters in the group with mandibular prognathism

Group P (n = 30)	NSAr	SNA	SNB	ANB	GoArNS
NSAr	1				
SNA	-0.567(**)	1			
SNB	-0.676(**)	0.674(**)	1		
ANB	-0.200	0.787(**)	0.076	1	
GoArNS	-0.230	0.161	-0.189	0.385(*)	1

NSAr – cranial base angle; SNA – maxillary prognathism angle; SNB – mandibular prognathism angle; ANB – angle of sagittal intermaxillary relationships; GoArNS – inclination angle of the ramus to the cranial base. *Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed).

In the present study, measuring the ST depth, as a normal distance from the line connecting clinoid extensions to the lowest point of the floor contours, a statistically significant difference was obtained between the patients with mandibular prognathism and eugnathic subjects. Namely, in the patients with mandibular prognathism, the ST depth was significantly higher (9.33 mm *vs* 7.55 mm, $p < 0.01$). Besides, the largest anteroposterior diameter used to present the ST width, was significantly higher in the patients with mandibular prognathism than in the eugnathic subjects (11.07 mm *vs* 9.53 mm, $p < 0.01$).

Discussion

The morphology of the cranial base is not the only factor which influences the formation of sagittal malocclusions, or the degree of its manifestation. In the literature, there are different and even opposing views on the interrelationship of the cranial base morphology and the size on the one hand, and sagittal malocclusions on the other one. These dilemmas have induced us to examine the dimensions of the cranial base and frontal facial part, trying to determine their influence on the development of profile in patients with mandibular prognathism. According to all the analyzed parameters of the aforementioned structures, it was found that only the dimensions of the *sella turcica* and nasal bone were different between the eugnathic subjects and the patients with mandibular prognathism.

The NAr, NS and SAr, NSAr showed lower values in the patients with mandibular prognathism than in the eugnathic subjects, but they were statistically insignificant. Although many authors [27–29] found that the cranial base angle was significantly reduced in mandibular prognathism, specifying it, as an important etiologic factor and one of the early indicators of anomaly development, some recent studies, where present results fit, suggest that there are changes of the size and shape of the cranial base, but they are not decisive. Thus, the relationship between the cranial base and malocclusions is much more complex [3, 5, 8]. The present study also detected a statistically negative correlation between the NSAr and the SNA and the SNB prognathism, which certainly indicated the connection between the cranial base angulation and sagittal intermaxillary relationships. Singh et al. [9] and Singh [28] have explained that the changed inclination of the posterior cranial base moves the condyle and the entire mandible anteriorly, whereas the changed inclination of the anterior cranial base is responsible for the maxilla repositioning, both resulting in Class III facial profile.

Anderson and Popovich [10] tried to establish the relationships between the cranial base size and shape and all the three skeletal class intermaxillary relationships. They found that the cranial base angle in skeletal Class III was smaller, and the condyle placed more anteriorly. Besides, they detected a strict correlation between the cranial base and maxilla length and a weak correlation with the mandible length. However, according to these authors, the size of maxilla does not have impact on prognathism, whereas the cranial base angle is in a strict correlation with the SNB angle, the angle of mandibular prognathism. Thus, they concluded that the size and shape of the cranial base influenced mandibular prognathism through the anteroposterior condylar position. In the present study, a statistically insignificant reduction of the angle of inclination of the ramus to the GoArNS in mandibular prognathism and a significantly positive correlation of this angle with the ANB were also found, indicating more anterior position of the temporomandibular joint. This finding indicates that the position of mandible depends on the morphological characteristics of the cranial base in the examinees of this study.

The results of morphogenetic tests conducted by Ellis and McNamara [27] showed that in skeletal Class III there were a significant bending of the cranial base, reduction of its posterior part and the angle between the ramus and the general cranial base plane. Singh [28] added that the glenoid fossa in skeletal Class III was placed more anteriorly than in Class I and II, which resulted in moving the temporomandibular joint anteriorly. Proff et al. [5] also found the reduction of the cranial base angle, its total length and posterior part in patients with mandibular prognathism. Their morphometric study suggests that a primary etiologic factor in the development of skeletal Class III might be an early ossification of petro-spheno-occipital complex synostosis, causing the insufficient horizontation of the cranial base and consequently anterior displacement of the condyle [30, 31].

Since the ST is a part of the cranial base, the cranial base shape and dimensions influence the ST position and dimensions. On the other hand, the cranial base shape and dimensions depend on the sagittal intermaxillary relationships [3]. This leads to the conclusion that the ST dimensions

and position also depend on the sagittal intermaxillary relationships. The clinical picture of mandibular prognathism actually shows abnormal intermaxillary relationships as its dominant symptom.

Examining the *sella turcica* dimensions in the patients with dentofacial deformities, several autors [18–20] found that all the three measured dimensions of *sella turcica* (surface, width and depth) were statistically much higher in the patients with deformities than in the eugnathic subjects, but the degree of the anomaly manifestation did not influence the size of changes in the aforementioned dimensions. Investigating correlations, they found that the depth of *sella turcica* had a positive correlation with the ST surface. This could be related to the fact that the ST floor, anterior and posterior wall are most susceptible to changes [14, 17, 24]. Alkofide [22] found that the largest anteroposterior diameter in patients with skeletal Class III was significally higher than in patients with other analyzed classes.

Having in mind that several studies have recently proved the increase in *sella turcica* dimensions in mandibular prognathism, it can be expected that future studies will find the cause and relationship between these phenomena [18–22].

The patients with mandibular prognathism also showed a significant increase in the nasal bone length. The nasal bone length and inclination to the cranial base in healthy subjects and patients with acromegaly were measured by Dostálová et al. [24], and they found that the nasal bone did not change its dimensions in the patients with acromegaly. In addition, these results showed that the average value of the nasal bone length in healthy women and men was similar approximately 23 mm, (in our study, the average value was 24.7 in the eugnathic subjects, whereas it was 27.58 mm in the patients with mandibular prognathism). The angle between the nasal bone and cranial base was 115° in the eugnathic subjects (in present study, the average value was 118.10° in the eugnathic subjects and there were no statistically significant differences between the analyzed groups).

Singh et al. [9] found a negative correlation between the frontonasal angle and the cranial base angle. In patients with skeletal Class III, the cranial base angle is actually reduced, so that the frontonasal angle is increased, resulting in a flat mid-face profile, which is a frequent characteristic of this dentofacial deformity. The angle of protrusion of the supraorbital ridge, which we measured, although reduced in mandibular prognathism, did not show any statistically significant differences between the groups.

As mentioned at the beginning, in both mandibular prognathism and developmental malocclusion, a significant increase in the mandible and change in mandibular shape occur during rapid growth at puberty. This change is primarily caused by the opening of the gonial angle, particularly characteristic for a hyperdivergent facial profile. It certainly results in changing the inclination of the ramus to the cranial base. The condylar cartilage is still active, therefore it is also very likely to have the remodeling growth of condyle and glenoid fossa changing their position and shape in this type of malocclusion [1, 2]. Thus, the inclination of the ramus to the cranial base causing the anterior mandibular positioning, does not strictly depend on the length of the posterior cranial base and its angulation, but most likely on other growth processes, such as the opening of gonial angle which occurs much later.

When discussing skeletal Class III, one should also think about mandibular prognathism and its developmental nature, which often camouflages by compensatory mechanisms (in rare cases potentiates) some important indicators in certain life phases. Therefore, the results of many studies are contradictory. It should not be forgotten that many growth and developmental studies have found that the mandible grows more intensively and longer through all life phases, even a year longer after the completion of general somatic growth [25, 26, 29]. Since the cranial base growth ends early, it can be considered as one of the etiologic factors of mandibular prognathism, but certainly not the decisive one, and although existing, the cranial base correlation with mandibular prognathism is not as simple as previously thought.

Conclusion

The results of this study show that the cranial base dimensions and angle do not play a significant role in the development of mandibular prognathism.

An interrelationship analysis indicated a statistically significant negative correlation between the NSAr and the SNA and the SNB prognathism, as well as a positive correlation between the GoArNS and the ANB.

Sella turcica dimensions, width and depth, as well as the nasal bone length were significantly increased in patients with mandibular prognathism, while the other analyzed dimensions of frontal part of the face were not changed by the malocclusion in comparison with eugnathic patients.

The impact of the cranial base and frontal part of the face on facial profile in patients with mandibular prognathism is much smaller, but certainly more complex than previously thought, and therefore it suggests, that morphogenetic tests of the maxillomandibular complex should be included in further assessment of this impact.

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Resection or radiofrequency ablation of colorectal liver metastasis

Resekcija ili radiofrekventna ablacija metastaze kolorektalnog karcinoma u jetri

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Abstract

Background/Aim. Liver resection is the treatment of choice for solitary colorectal liver metastases in suitable candidates. Recently, radiofrequency ablation (RFA) has become a very popular procedure in the treatment of liver metastases. The aim of this study was to compare outcomes in patients with solitary colorectal liver metastasis who had been subjected to resection or ablation. **Methods.** In this retrospective study we analyzed and compared patients with solitary colorectal liver metastases treated by resection or ablation in the University Hospital Centre "Dr Dragiša Mišović" in Belgrade from January 2002 until December 2009. **Results.** In this study 94 (67.1%) patients underwent resection whereas 46 (32.9%) patients underwent RFA. Most of the resected patients (59.6%) required major hepatectomy. The median follow-up time was 28.4 months. Tumor ablation was a significant predictor of the overall survival (p = 0.002; OR 3.75; 95% CI 1.696–8.284). Our study demonstrated longer disease free-survival in the group of resected patients compared to the RFA group (37.6 *vs* 22.3 months, p = 0.073). The median overall survival was 56.3 months for patients who underwent resection *vs* 25.1 months for those in the RFA group (p = 0.005). **Conclusion.** This study shows that the patients with solitary hepatic colorectal cancer metastases should be considered for hepatic resection whenever it is feasible, because this procedure provides superior long-term survival as compared to radiofrequency ablation.

Key words:
colorectal neoplasms; digestive system surgical procedures; liver neoplasms; neoplasm metastasis; catheter ablation, treatment outcome.

Apstrakt

Uvod/Cilj. Hirurška resekcija jetre predstavlja metod izbora u lečenju pojedinačnih metastaza kolorektalnog karcinoma u jetri kod odgovarajućih bolesnika. Radiofrekventna ablacija postaje sve popularnija metoda za lečenje metastaza u jetri. Cilj ove studije bio je da uporedi ishode bolesti kod bolesnika sa pojedinačnom metastazom kolorektalnog karcinoma u jetri koji su lečeni hirurškom resekcijom u odnosu na bolesnike koji su lečeni radiofrekventnom ablacijom (RFA). **Metode.** U ovoj retrospektivnoj studiji analizirani su bolesnici sa pojedinačnom metastazom kolorektalnog karcinoma u jetri koji su lečeni u KBC „Dr Dragiša Mišović" u Beogradu u periodu od januara 2002. do decembra 2009. godine. Poređeni su ishodi bolesti nakon hirurške resekcije jetre i nakon RFA metastaza u jetri. **Rezultati.** Studijom je bilo obuhvaćeno 94 (67,1%) bolesnika podvrgnutih resekciji jetre, dok je 46 (32,9%) bolesnika lečeno radiofrekventnom ablacijom. Kod većine bolesnika (59,6%) podvrgnutih hirurškoj resekciji učinjena je *major* hepatektomija. Prosečna dužina praćenja bolesnika bila je 28,4 meseca. Utvrđeno je da RFA tumora predstavlja značajni prediktor dužine ukupnog preživljavanja (p = 0,002, OR 3,75, 95% CI 1,696–8,284), te da je duže preživljavanje bez tegoba bilo u grupi bolesnika sa resekcijom u poređenju sa RFA grupom (37,6 *vs* 22,3 meseca, p = 0,073). Prosečno ukupno preživljavanje iznosilo je 56,3 meseca u grupi bolesnika sa hirurškom resekcijom naspram 25,1 mesec u RFA grupi (p = 0,005). **Zaključak.** Kod odgovarajućih bolesnika sa pojedinačnom metastazom kolorektalnog karcinoma u jetri trebalo bi razmotriti hiruršku resekciju kad god je to izvodljivo, jer pruža duži period preživljavanja nego lečenje radiofrekventnom ablacijom.

Ključne reči:
kolorektalne neoplazme; hirurgija digestivnog sistema, procedure; jetra, neoplazme; neoplazme, metastaze; ablacija preko katetera; lečenje, ishod.

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Introduction

Colorectal cancer (CRC) remains one of the leading causes of mortality caused by malignancy. Approximately 25% of all colorectal cancer patients at the time of initial diagnosis already have liver metastases, and additionally 50% will develop distant metastases in the next 5 years [1]. Treatment of colorectal cancer patients with metastases on the liver is a therapeutic challenge and requires multidisciplinary treatment. Nevertheless, surgery is the treatment of choice for these patients. Survival data shows that with modern, multidisciplinary treatment, 25–60% of patients with liver resection to treat CRC metastases survive more than 5 years [2–7]. The goal of operation is to remove all metastatic tumor tissue with acceptable resection margins. Some studies show that narrow margins do not have influence on survival, and that complete removal of metastases with minimal margins can be acceptable when it is not possible technically to obtain wider margins [8–10]. Due to the importance of liver disease reduction in cases with metastases that cannot be resected, new methods of local treatment of metastases are developed, among which is radiofrequency ablation (RFA). RFA uses thermal energy produced by radiofrequency generator to destroy tumor and a small part of surrounding healthy tissue [1, 3, 4, 11]. The five-year survival rate after RFA in different studies ranges from 14% up to 27% [1, 3, 5, 11–13].

Methods

The study is a retrospective analysis of patients with solitary CRC liver metastases treated with RFA or surgical resection, in the University Hospital Centre "Dr Dragiša Mišović" in Belgrade, from January 2002 until December 2009. Metastases are considered resectable when it is possible to remove the tumor with negative resection margins, leaving functionally sufficient liver tissue. The patients with extrahepatic metastases are excluded from this study. RFA was performed with open approach after laparotomy to all patients in this group, and the criteria for RFA were unresectability of metastases and comorbidity (accompanying diseases and conditions), which significantly increased the risk of liver resection. Data about chemotherapy were not known for all the patients, and for most were not reliable, so these were not considered in this study. The patients treated with RFA were compared with the patients treated by liver resection by using *t*-test, χ^2-test and Fisher's exact test where appropriate. Statistical analysis was performed by using JMP 4.0 and SPSS version 16 software. Continuous variables were compared using Student's *t*-test, and categorical variables were compared by using χ^2-test. The survival was plotted by Kaplan-Meier method, and compared using the *log-rank* test. A value $p < 0.05$ was considered significant. The overall survival was calculated from the moment of diagnosis until death. Cox regression method was used in order to establish independent predictors of disease outcome. Multivariate analysis was performed with the Cox's proportional hazards model.

Results

A total of 140 patients with solitary CRC liver metastases were indentified from the database of operated patients in the University Hospital Centre "Dr Dragiša Mišović" in Belgrade within the period from January 2002 until December 2009. The median follow-up time was 28.4 months. The median age of the patients was 62.9 years, among which were 74 (52.9%) male and 66 (47.1%) female patients.

Primary tumor localization was mostly on the left colon and rectum, and most often localization was the sigmoid colon with 32.9%, then cecum and ascending colon with 31.4%, the rectum with 21.4%. In 10% of the patients, primary localization was unknown. Most of the patients had locally advanced primary tumor, 72.9% with T3 stage, and 60% of the patients had regional lymph nodes metastasis during the initial operation of the colon. Synchronous metastases in the liver were seen in 66 (47.1%) of the patients (Table 1).

Liver metastases were resected in 94 (67.1%) of the patients, while in 46 (32.9%) of the patients RFA was performed. The majority of resected patients (59.6%) underwent major hepatectomy. The most often anatomic resection was right hepatectomy (29.8%) then left hepatectomy (12.8%) and extended right hepatectomy (12.8%). Extra-anatomic "wedge" resections were represented with 12.8% (Table 2).

Table 1

Primary and metastatic tumors characteristics

Tumor characteristics	n	%
Depth of primary tumor invasion		
T1	2	1.4
T2	10	7.1
T3	102	72.9
T4	12	8.6
unknown	14	10.0
Primary tumor localization		
cecum and ascending colon	44	31.4
transverse colon	4	2.9
descending colon	2	1.4
sigmoid colon	46	32.9
rectum	30	21.4
unknown	14	10.0
Lymph node involvement (N1)	84	60.0
Synchronous primary tumor and metastasis	66	47.1

Most of the patients (89.4%) had R0 resection, with the median resection margin of 1.8 cm.

Table 2

Type of liver resection

Type of resection	n	%
Right hepatectomy	28	29.8
Left hepatectomy	12	12.8
Extended right hepatectomy	12	12.8
Extended left hepatectomy	4	4.3
Central liver resection	2	2.1
Left lateral segmentectomy	10	10.6
Right posterior segmentectomy	6	6.4
Resection of one segment	8	8.5
Wedge resection	12	12.8

Comparing the patients with RFA and those with resection showed no significant difference regarding sex (p = 0.632), age (p = 0.992) and lymph node involvement (p = 0.368) in these two groups. The patients in the resection group had significantly larger metastases in the liver (5.5 cm in relation to 3.85 cm in the RFA group, p = 0.004) (Table 3).

The median disease free survival was 37.6 months for the group of resected patients, and for the RFA group it was 22.3 months (p = 0.073). The median overall survival was 56.3 months for the resected patients, while for the RFA group it was 25.1 months (p = 0.005). There were no significant predictors of recurrence using univariate analysis. Age, T-stage, N-stage, resection margins, size of metastasis and RFA individually did not affect the recurrence rate (Table 4).

Table 4

Local recurrence predictors

Factor	Univariate p-value
RFA *vs* resection	0.07
Metastasis size	0.092
Lymph node involvement (N1)	0.20
Age	0.557
Gender	0.544
Tumor invasion (T-stage)	0.663
Primary tumor localization	0.910
Resection margin	0.569

RFA – radiofrequent ablation.

The significant difference regarding a shorter overall survival period in the RFA group was established using multivariate analysis (p = 0.002; OR 3.75; 95% CI 1.696–8.284). Age, T-stage, N-stage, resectional margin and size of metastasis did not individually affect the overall survival period (Table 5).

From the RFA group 16 (34.8%) patients developed local intrahepatic recurrence. In 6 (13.0%) of the patients, recurrence was on the ablation site or its margin. In the group of resected patients, 12 (12.8%) patients developed local intrahepatic recurrence (p = 0.026). One of them had recurrence on the resection area. There were no significant differences in the occurrence of extrahepatic metastases between the RFA and the resection group (21.7% and 18.9%, respectively; p = 0.2).

There were no significant differences in the postoperative complications rate (p = 0.35) between the two groups. In the group of resected patients, 23 (48.9%) had complications

Table 3

Demographics and tumor characteristics of the patients

Characteristics	RFA	Liver resection	p-value
Gender (male), (%)	52.2	51.1	0.632
Age (years)	62.2	60.8	0.992
Size of liver metastases (cm)	3.8	5.5	0.004
Lymph node involvement (N1), (%)	52.17	63.83	0.368
Depth of primary tumor invasion (T-stage)			0.11
T1	0	2	
T2	2	8	
T3	34	68	
T4	4	8	
unknown	6	8	
Synchronous metastasis (%)	43.48	48.94	0.627

RFA – radiofrequent ablation.

Table 5

Overall survival predictors

Factor	Univariate p value	Hazard ratio	95% CI
RFA *vs* resection	0.006	2.5	1.3–4.8
Age	0.407		
Gender	0.558		
T stage	0.995		
Primary tumor localization	0.946		
Resection margin	0.330		
Size of metastasis	0.975		
Lymph node involvement (N1)	0.842		

RFA – radiofrequent ablation; CI – Confidence interval.

of any kind, while in the RFA group, 18 (39.1%) of the patients had complications. Nevertheless, complications in the RFA group were minor, mostly prolonged fever, while in the group of resected patients major complications were registered. There was one (1.1%) postoperative death in the group of resected patients. A patient with metastasis size 6 × 7 cm, located between the right and the middle hepatic vein, underwent extended right hepatectomy. Because of liver bleeding, the patient was reoperated after 18 h, and adequate hemostasis was accomplished. On the fourth postoperative day the patient died from massive myocardial infarction. In the RFA group, there was no postoperative mortality.

Discussion

Surgical resection of CRC liver metastases is not possible for many patients despite indisputable improvement of surgical technique [14, 15]. The main limiting factors are anatomic localization of metastases, functionally insufficient remnant liver, extensive comorbidity or the presence of extrahepatic metastases [16]. Resection could not be performed in cases of metastases invading the portal vein, hepatic artery or the cava vein, or the portal vein thrombosis [17]. Several studies show that patients with unoperated liver metastases have a low 5-year survival rate [12, 15]. Patients with CRC liver metastases treated only with chemotherapy have poor prognosis with the approximate median survival of 21 months, despite its enormous improvement [2]. The most usual cause of death is the progression of liver illness, leading to liver insufficiency.

Some patients are not ideal candidates for the liver solitary metastases resection, even though that is the method of choice. Furthermore, the incidence and severity of complications after RFA are more acceptable to surgeons than those after resection of the liver. These factors have, among others, increased popularity of RFA in treatment of liver metastases. Despite its attractiveness, RFA can give worse results than resection to patients that could be operated [3].

We tried to establish if there is a significant difference in the outcome for patients with solitary CRC liver metastases, treated by resection or RFA. Our data show that resection of the liver is superior to RFA. Therefore, we believe that resection of CRC liver metastases remains the method of choice for solitary lesions on the liver. Due to patients who are not suitable candidates for hepatectomy, new "less invasive" methods for treatment of liver metastases are promoted. One of the most popular methods is thermal ablation of metastases using radiofrequency power. During previous few years, RFA was suggested as alternative for surgical resection for patients with CRC metastases on the liver. In 2003, Oshowo et al. [4] published that the 3-year survival was 55% for patients with solitary metastases in the liver that were resected, while in the group where solitary metastases were treated by RFA, survival was 53%. In that study, RFA was used only for patients considered unsuitable for surgical resection. This selection was made in our study as well, because surgical opinion in our hospital is that CRC liver metastasis resection has to be done whenever it is possible, and RFA is reserved for patients with non-resectable metastases, or have other severe accompanying diseases that significantly increase the risk concerning extensiveness of liver surgery.

Our study showed a longer disease free survival in the group of resected patients comparing to the RFA group (37.6 *vs* 22.3 months, respectively; $p = 0.073$). Many factors are related with higher risk of recurrence after treatment of CRC metastases in the liver. Most commonly described factor is the size of metastases. In our study, the size of metastases was significantly larger in the group of resected patients than in the RFA group. A recent study has shown that the incidence of local recurrence increases by 33% after RFA of metastases larger than 3 cm [2]. Aloia et al. [5] have analyzed patients with solitary colorectal metastases in the liver and compared patterns of local recurrence after hepatectomy and RFA. They have established that RFA was associated with the high (37%) incidence of local recurrence, unrelated to the size of metastases, and with shorter disease-free survival and survival period. Our study showed a significantly higher incidence of local recurrence in the group of patients with RFA (34.8%), compared to the resection group (12.8%). A recurrence rate after RFA in our study was similar to those published in recent studies [5, 11]. Unfortunately, repeated treatment of local recurrence with RFA is often impossible or unsuccessful [12, 18]. That is the reason why we believe that resection of the liver should be offered to all patients with resectable CRC liver metastases.

We found no significant differences between the two groups of patients in characteristics of primary tumor, including depth of tumor invasion (T-stage), involvement of lymph nodes (N-stage) or synchronicity of metastases. Despite similarities between the two groups in traditional predictors of survival, liver resection showed significantly better results. The only statistically significant predictor of the overall survival was the type of treatment (resection or RFA). The differences in the disease-free survival and the overall survival between the resected and RFA patients cannot be explained by differences in demographic characteristics, characteristics of primary tumor, characteristics of metastases or other perioperative factors. That proves oncological superiority of resection comparing to RFA.

Abdalla et al. [3] have published a retrospective analysis of patients with CRC liver metastases submitted to resection, RFA or a combination of these two methods. They have shown a 4-year survival of 65% in the group of resected patients, while in the RFA group it was 22%. Interesting fact is that this percent in the group of patients submitted to both resection and RFA was only 36%. These data show that RFA individually, or in combination with resection, does not provide the length of survival comparable with that after resection of the liver in treatment of CRC liver metastases. RFA was used in treatment of solitary metastases localized on the places impossible to leave a negative resection margin. In their study, there is a statistically significant difference in survival between patients submitted to resection and those submitted to RFA ($p = 0.025$) [3]. These data support our results that the median

survival of the patients in the resection group is significantly longer than in group treated with RFA.

There are certain limitations of our study. Firstly, this is a retrospective study with some unknown data. Secondly, resection was done to our patients wherever it was possible, leaving RFA for unresectable metastases or for those with seriously damaged health by comorbidities. It is possible that those factors contributed to the difference in the duration of survival. Finally, not all the patients in this study were treated by chemotherapy, and those who were did not receive identical protocols, and we did not have data about chemotherapy of a certain number of patients. Therefore, the conclusions of this study should be interpreted with some caution.

Conclusion

Patients with solitary CRC liver metastases should be considered for surgical liver resection whenever it is feasible, because it provides a long-term survival compared to treatment with radiofrequency ablation. This study promotes aggressive resection of solitary liver metastases, because RFA is associated with a shorter disease free-survival and a shorter overall survival.

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Surface enlargement of a new arterialised venous flap by the surgical delay method

Povećanje površine novog arterijalizovanog venskog režnja metodom hirurškog odlaganja

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Abstract

Background/Aim. The delay method is a surgical, pharmacological and combined method that includes two or more time separated phases, which gives bigger flap surface. In our research we explored the possibility of flap surface enlargement in a new arterialised venous flap (AVF) on an experimental rabbit ear model by the delay surgical method. The aim of this research was to establish vitality surface of our AVF and to maintain the difference in flap vital surface between AVF flaps, with or without performing the delay surgery method. **Methods.** We used both ears of "Big Chinchilla" rabbits in 10 experimental male animals, divided into two groups, average weight 3–3.5 kg, and average age 8–10 months. In the first (experimental) group, a venous flap was arterialised by our method. In the second (control) group, the venous flap was arterialised 14 days after the delay surgical method. AVF surface was measured on the 1 and 14 days by the method of trapezoid rule. **Results.** Vital surface on our AVF experimental model was bigger than 87% of elevated flap surface after the delay surgical method. Vital surface on AVF without delay on our experimental model was bigger than 30% of elevated flap surface ($p < 0.001$). **Conclusion.** Analysis of previous experimental models on the rabbit ear, non-delayed and delayed (to enlarge flap surface) led us to conclusion that previously created experimental models of non-delayed AVF are hemodynamically negative. Our experimental non-delay AVF model is hemodynamically more positive than previously created models of non-delay AVF and provides better conditions for AVF survival and enlargement of vital flap surface of elevated flap. On the other hand, surgical delay method significantly enlarges vital surface of AFF.

Key words:
surgical flaps; reconstructive surgical procedures; methods; rabbits.

Apstrakt

Uvod/Cilj. Metoda odlaganja je hirurška, farmakološka i kombinovana metoda koja obuhvata dve ili više vremenski razdvojenih faza, što daje veću površinu režnju. U istraživanju je ispitivano povećanje površine novog arterijalizovanog venskog režnja (AVR) na eksperimentalnom modelu uha kunića metodom hirurškog odlaganja. Cilj rada bio je da se utvrdi vitalna površina našeg AVR i utvrdi da li postoji razlika u veličini vitalne površine AVR sa ili bez primene metode hirurškog odlaganja. **Metode.** U eksperimentalnom istraživanju upotrebljena su oba uha kunića rase *Big Chinchila* u 10 eksperimentalnih životinja muškog pola, težine od 3–3,5 kg, starosti 8–12 meseci podeljenih u dve grupe. U prvoj (eksperimentalnoj) grupi, arterijalizovan je venski režanj po našoj metodi, a u drugoj (kontrolnoj) grupi arterijalizovan je venski režanj nakon 14 dana od metode hirurškog odlaganja. Površina AVR određivana je 1. i 14. dana metodom trapezoidnog pravila. **Rezultati.** Vitalna površina AVR na našem eksperimentalnom modelu nakon metode hirurškog odlaganja bila je veća od 87% površine odignutog režnja. Vitalna površina AVR koja nije odložena na našem eksperimentalnom modelu iznosila je više od 30% površine odignutog režnja. ($p <$ 0,001). **Zaključak.** Analizom dosadašnjih eksperimentalnih modela AVR na uhu kunića, neodloženih i onih kod kojih je radi povećanja vitalne površine primenjena metoda odlaganja režnja, utvrdili smo da su dosadašnji kreirani modeli neodloženih AVR hemodinamski nepovoljniji. Naš eksperimentalni model neodloženog AVR je hemodinamski povoljniji od prethodno kreiranih modela neodloženog AVR i omogućava povoljnije uslove za preživljavanje AVR i povećanje vitalne površine odignutog režnja. S druge strane, metodahirurškog odlaganja značajno povećava vitalnu površinu AVR

Ključne reči:
režnjevi, hirurški; hirurgija, rekonstruktivna, procedure; metodi; zečevi.

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Introduction

Delay flap methods have been used for many centuries in various models, but remained not completely pathophisiologically explained until today. The term "delay phenomenon" was introduced by Blair [1] in 1912 for decryption of preliminary stages of flap elevation, and is used until today. In the 16th century Gaspare Tagliacozzi delayed brachial flap, making parallel incisions in skin and subcutaneous tissue for nose reconstruction [2].

The delay method is a surgical, pharmacological and combined method that includes two or more time separated phases, which gives bigger flap surface.

In the first phase, the skin and subcutaneous tissue are cut *via* marked lines of a planed flap. The aim of delay in this phase is to interrupt peripheral circulation, for making independent orientation of blood vessels in the part of connected tissue which was a flap basis.

In the second phase, after 10 to 14 days, the flap is completely divided (except flap basis) from undersurface and moved into defect. This is an optimal period in the surgical delay flap method, confirmed by Cheng et al. [3], Kelly et al. [4], Kushima et al. [5] and used in this research, too.

Arterialized vein flap (AVF) delay was demonstrated for the first time by Nakayama et al. [6] in 1981, in epigastric skin flap in rats. The results were confirmed in a similar study by Voukidis [7].

Another researches were directed to arterialisations of venous flap without previous delay (so-called acute arterialization of venous vessel space) [8]. Nichter and Haines [9] developed experimental AVF model on rabbit ear, making arteriovenous communication side-to-side, between the artery and vein, preserving both marginal vein for drainage.

Usage of central vein path and preservation of symmetrical marginal drainage veins increases survival rate of an AVF [10–12].

To clear up some of dilemmas, we studied that situation, discussing the delay surgical method in AVF. The aim of this study was to explore the possibility of surface enlargement in an AVF using the delay surgical method of flap.

In this work, we introduced an original experimental AVF model hemodynamically better than the previously demonstrated delay surgical method of flap [11].

In the first (experimental group) of study animals, AVF was applied by the surgical method.

In the second (control group) of experimental animals, AVF was applied by the delay surgical method.

We measured and compared AVF in both groups of animals, and, according to the results, we established the difference in size between surfaces of AVF performing one or another method.

Methods

In this experimental research we used both ears of "Big Chinchilla" male rabbits, weight 3.2–4.5 kg, age 12–18 months, from the experimental animals farm of Military Medical Academy in Belgrade (Figure 1).

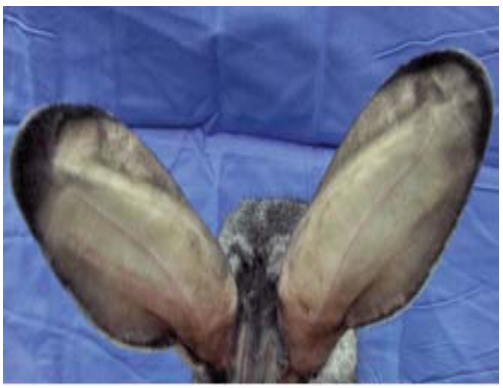

Fig. 1 – Experimental model.

Before the experiment, animals were kept in the vivarium of the Institute for Medical Research of Military Medical Academy, Belgrade, for the period of adaptation. Animals were nourished and watered "*ad libidum*".

For intravenous anesthesia, we used ketamine chloride in the dosage of 35 mg/kg of body mass, acepromazine maleate 1 mg/kg of body mass in 500 mL 0.9% NaCl and 100 IU heparine *per* kg of body mass. An intravenous cannula with 0.5 mm diameter was placed in the anterior marginal vein, and with infusion, drop by drop, anesthesia was maintained for approximately 4 h (Figure 2).

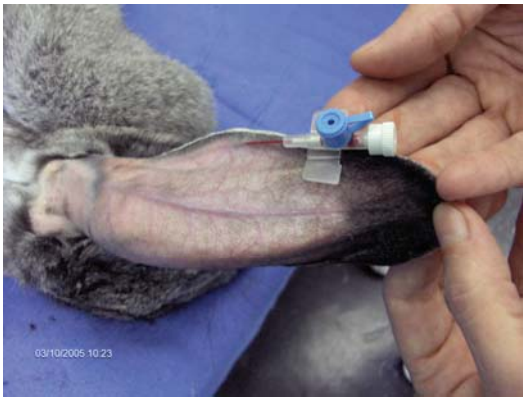

Fig. 2 – Intravenous cannula at site.

First, incision on the skin and subcutaneous tissue was done under the communicant vein, and access made to the vascular pedicle, thus preparing blood vessels. Emptiness test of prepared vessels was used to determine anatomic position of the central artery and vein. Central nerve and blood vessels were cut. A distal part of central vein and proximal part of central artery were tied. After establishing end-to-end anastomosis, we used Acland test to estimate transience, and then the ear was cut at all levels, with preservation of anterior marginal vein in which was intravenous cannula placed. Wound was sutured on anatomical layers. In this way, arterial blood supplied through venous vessel net a block of tissues, consisting of skin, subcutaneous tissue and cartilage. Preserved anterior marginal vein represented outflow vessel of AVGF. Flap vitality was estimated on the days 1 and 14, recording all parameters in experimental protocol for each animal (Figure 3). All animals were treated with heparine in

the dosage of 100 IU *per* kg of body mass during 14 postoperative days. To prevent infection, all animals were treated with 100 mg oxitetracycline in a single daily dosage.

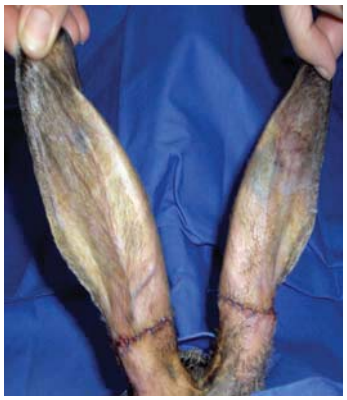

Fig. 3 – Arterialised vein flap formation.

Five experimental animals were used and an AVF was applied to both rabbit ears (10 samples).

The ear was cut at all levels, preserving the anterior marginal vein (Figure 4). The microsurgical technique was

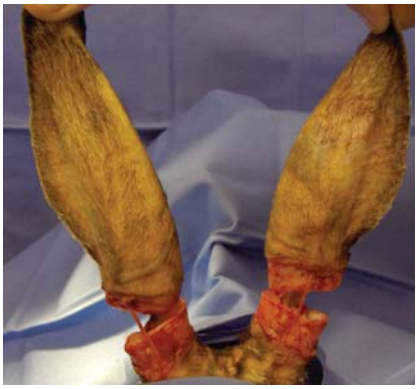

Fig. 4 – Arterialised vein flap preparation.

performed under 20 times of magnification, and the central artery and the central vein were anastomosed by single stitches (Figure 5). For the microsurgical end-to-end anasto-

Fig. 5 – End-to-end arterial-vein anastomosis.

mosis technique we used ethilon 10.0 monofilament polyamide black suture material, with a nontraumatic rounded curved needle 3.75 mm long, and 75 μ in diameter, manufactured by Ethicon Ltd. Blood vessels were tied with surgical thread monofilament polypropylene prolene blue 6–0, and wound was sutured with prolene blue 3–0 by single stitches, manufactured by Ethicon Ltd.

The same number of experimental animals (5) was used and before vein flap arterialisation the surgical delay method was performed on both rabbit ears also, so we had the same number of samples (10 samples).

Ear skin was cut only (Figure 6). Subcutaneous blood vessels and the central nerve were identified and all cut (Figure 7) except the anterior marginal vein, central artery and central vein, which still nourished rabbit ear. After 14 days from performing the surgical delay method (Figure 8), arterialisation of a venous flap was done by microsurgical end-to-end anastomosis technique, using the previously explained procedure.

Fig. 6 – Ear skin incision.

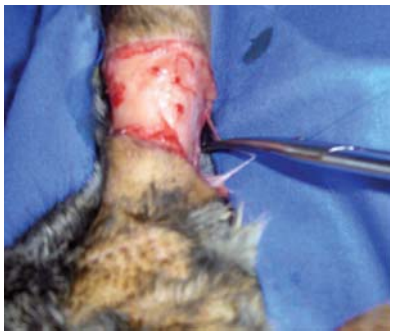

Fig. 7 – Cut of the nerve.

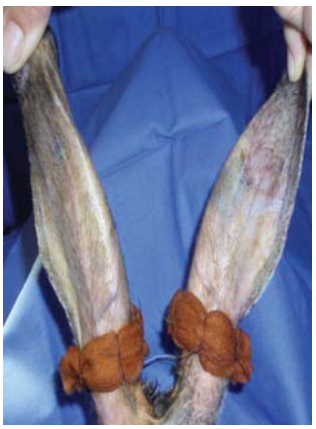

Fig. 8 – Delayed arterialised vein flap.

For surgery we used a binocular operational microscope OPTION I, with limited magnification of 5 to 25 times, with 200 mm focus optical distance. Standard microsurgical instruments and the above mentioned suture material were used.

The experimental animals were numbered previously and marked by experimental groups in experimental protocol.

After arterialisation of the venous flap, clinical estimation of microanastomosis transience was done. Early transience was evaluated by inspection and Acland test, immediately after microanastomosis was completed, exactly after 20 minutes. Microanastomosis transience was evaluated after 24 h by palpation and enlightening technique of the rabbit ear using neon light.

Palpable pulsations in regular time interval and fast fullfillness of blood vessels after vessel emptiness, were treated as a previous sign of clinical anastomosis. Previous anastomoses were included in further investigation.

AVF surface size in the experimental groups, on the days 1 and 14 were defined as: flap length in cm, measured by the longest axis; flap wideness in cm, measured by the widest axis; flap surface in cm^2.

Flap surface was measured after previously marked flap borders on a transparent foil with regions of flap necrosis (Figure 9). The marked surfaces were measured by computer program with integrals and trapezoid rules, as well as statistical program set. After the described process, transparent foils were put in the experimental animal evidence documentation.

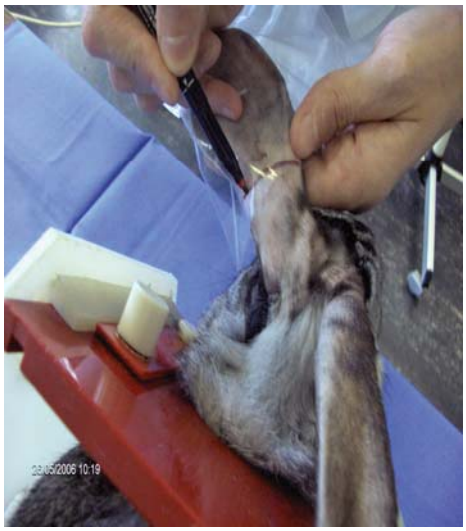

Fig. 9 – Marking flap surface borders on transparent foil.

Necrosis surface was defined as a region of full thickness of the rabbit ear without bleeding (sterile needle test) (Figure 10), as well as surfaces which did not epithelise and were covered by crusts. Measurements were performed on the days 1 and 14 after venous flap arterialisation in both groups.

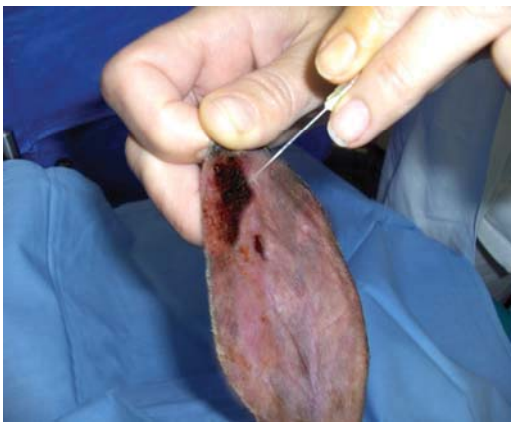

Fig. 10 – Sterile needle test.

Statistical methods

For statistical evaluation of the differences between some characteristics of experimental groups, the examined parameters were presented as mediana, minimal and maximal values, standard deviation (SD) and coefficient of variation (CV).

A statistical significance between some characteristics of experimental groups was defined by the Student *t*-test for independent samples.

The 3 levels of statistical significance were formed: $p < 0.05$, $p < 0.01$ and $p < 0.001$.

The commercial statistical software for PC (Stat for Windows, R.4.5, Stat Soft, Inc., SAD, 1993) was used.

All procedures were done according to the ethic principals of scientific research work on experimental animals in Military Medical Academy, Int. No. 282-12 from December 20, 2002.

Results

The final results of both groups of experimental animals in which venous flap arterialisation was done without the surgical delay method (group I) or with the surgical delay method (group II), are presented in Table 1.

Table 2 shows the surface of both sides of AVF in cm^2, surface of necrosis in cm^2, and a total percentage of necrosis and survival tissue AVF. Measurements were done on the days 1 and 14 after vein system arterialisation.

Discussion

According to the experimental research, the delay method in axial flap can be performed by one of the mentioned ways: artery, vein and nerve cutting; nerve cutting with arterial and vein adventitia cut; artery and vein cutting with adventitia preservation; cutting only artery with the biggest preservation of adventitia; cutting only vein (adventitia preserved).

According to the traditional Krough method, oxygen transportation is done through terminal arterioles. During blood circulation through the lungs, carbon dioxide diffuses into alveoli. That decreases carbon dioxide partial pressure in

Table 1

Characteristics of arterialsed venous flaps in the group without (I) and with (II) the surgical delay method

Studied group	Parameters	Days after surgery					
		1			14		
		$\bar{x}$ ± SD	min–max	CV (%)	$\bar{x}$ ± SD	min–max	CV (%)
I	Left ear						
	viable surface (cm^2)	111.42 ± 11.32	99.29 –125.35	10.16	65.59 ± 12.44	52.13–80.27	18.96
	necrotising surface (cm^2)				45.83 ± 6.06	38.92–52.45	13.08
	necrosis (%)				41.13 ± 6.76	31.54– 52.38	16.16
	Right ear						
	viable surface (cm^2)	102.43 ± 9.14	90.28–112.57	8.92	63.41 ± 8.46	51.07–73.12	13.34
	necrotising surface (cm^2)				39.01 ± 1.58	36.29–40.18	4.05
	necrosis (%)				38.17 ± 6.35	26.77–48.99	16.63
	Total						
	viable surface (cm^2)	106.92 ± 10.80	90.28 ± 125.35	10.10	64.50 ± 10.10	51.07–80.27	15.65
	necrotising surface (cm^2)				42.66 ± 5.68	36.26–52.45	13.31
	necrosis (%)						
II	Left ear						
	viable surface (cm^2)	106.13 ± 11.52	95.54–120.11	10.85	96.85 ± 10.72	86.01–111.73	11.06
	necrotising surface (cm^2)				9.28 ±2.09	6.52–12.24	22.52
	necrosis (%)				8.58 ± 3.31	4.49–13.38	38.57
	Right ear						
	viable surface (cm^2)	99.98 ± 7.98	91.18–109.43	7.98	91.23 ±7.95	79.53–99.01	8.71
	necrotising surface (cm^2)				8.75 ± 2.63	6.07–11.64	30.05
	necrosis (%)				8.76 ± 3.80	4.08–15.45	43.37
	Total						
	viable surface (cm^2)	103.06 ± 9.89	91.18–120.11	9.59	94.04 ± 9.37	79.53–111.73	9.96
	necrotising surface (cm^2)				9.01 ± 2.25	6.07–12.24	24.97
	necrosis (%)				8.67 ± 3.47	4.08–15.45	40.02

$\bar{x}$ – average flap surface; SD – standard deviation; min – minimal; max – maximal; CV – coefficient of variation.

Table 2

Tissue survival on the days 1 and 14 after arterialisation of venous flaps on both rabbit ears surfaces

Parameters	Flap surface			
	1st day		14th day	
	anterior	posterior	anterior	posterior
Surface (cm^2), $\bar{x}$ ± SD	55.50 ± 5.38	51.42 ± 5.77	22.02 ± 3.66	20.64 ± 4.11
Necrotising surface (cm^2),			33.48 ± 4.85	29.78 ± 4.62
$\bar{x}$ ± SD [%]			[39.89 ± 6.98]	[40.11 ± 6.70]
Survival tissue (%), $\bar{x}$ ± SD			60.11 ± 6.97	59.89 ± 6.97

$\bar{x}$ – average flap surface; SD-standard deviation.

blood, as well as hydrogen ions with decreases of carbon acid concentration. Both factors move oxyhemoglobin dissociation curve to the left. That increases oxygen amount, which bonds to hemoglobin on that partial pressure level, and increases oxygen amount for tissues. When that blood reaches the tissue capillary net, a completely different phenomenon appears. Carbon dioxide leaving tissue moves dissociation curve to the right, facilitating oxygen release from oxyhemoglobin, which improves tissue supply with more oxygen compare with situation in oxygen release during that partial pressure in blood. This phenomenon is known as Bohr`s effect and is the key for understanding non-conventional circulation [12]. Noreldin et al. [13] experimented on rats inferior epigastic flap and discussed about small number of capillaries in areolar tissue that surround vein during flap vein elevation. They think that this periareolar tissue can give enough arterial blood for survival. They concluded that monopedicle vein flap survive primary on small arterial inflow from arterial plexus that surrounds vein pedicle. There is an opinion that any peri-vein vessel that is included in nutritional vein pedicle (arteriole, capillary loop, small vein) can bring enough oxygen to flap, if oxygen partial pressure in blood is higher than oxygen partial pressure in flap. According to the ischemic state of flap at the beginning, it is expected that periareolar blood vessels provide gas diffusion in flap until arterial neovascularisation. On the AVF example, this survival mechanism is not adequate. Vein mono- and bipedicle flap is completely separated from surroundings, and with vein separation completely transferred as free, and with microanastomosis between the recipient artery and flap vein, circulation in a flap is “reconstructed”. So, periareolar blood vessels are initially cut.

At the beginning of ischemic state, the flap is nourished by plasmatic imbibition's mechanism, using diffusion of oxygen from a recipient bed, with higher oxygen partial pressure, as in the case of free tissue transfer. These mechanisms provide gas diffusion and oxygen usage on precapillary level, until conventional neovasularisation is established [14].

The most of today's theories about survival mechanism of nonconventional flaps, consider that gas transportation on the capillary bed is essential for flap vitality until neovascularisation happen. In explaining blood flow through capillary system, the "rolling" mechanism of blood is considered, in regard to turns of blood flow forming. In total vein and AVF, blood enters capillary bed with the help of the already existing arteriovenous anastomosis.

Harris et al. [15] suggested that because of the parallel arrangement of bigger arterioles and venules in skeletal muscles, where arteriovenous anastomoses are less developed, mutual oxygen exchange appeared. They supported that diffusion between arterioles and venules can decrease oxygen partial pressure in tissue and increase in venulas. This mutual exchange is considered harmful for oxygen delivery. According to that theory, a part of oxygen in arterial blood diffuses in vein blood, making diffusion gradient in shunt. In the presence of Borh`s effect, mutual exchange increases oxygen partial pressure in tissues, according to mathematical models. This effect is especially emphasized in hypoxia, which is intensified by lactic acidosis. During hyperoxaemia, Borh`s effect does not have a big influence on oxygen partial pressure, because oxihemoglobin is completely saturated. Because of that, mutual oxygen exchange behaved as oxygen diffusion shunt, decreasing oxygen partial pressure and preventing toxic influence of hyperoxaemia. Mutual gas exchange, according to the previously explained diffusion gas transport, can be the another mechanism which helps in surviving of nonconventional miocutaneous flaps in the initial ischemic state without relying on the exchange between oxygen and carbon oxide on the capillary level [16].

Clinical appearance of AVF at the beginning is characterized with edema and congestion which withdraw slowly, between the postoperative days 5 and 10. Congestion is understandably the result of neovascularisation and the vascular net adaptation to the flap. Blood vessels neovascularisation is inducted by hypoxia, and adaptation by arteriovenous shunts opening and pressure increase in vein vascular net. Oscillated veins play a big role in these processes [17].

On our experimental model this phenomenon was also seen. The vein system is not anatomically adapted to high blood pressure. In newly developed conditions, filtration pressure on the ending part of venous net is increased. Exudation of albumins and other proteins through capillary fenesters in interstitial happens. Protein fractions moved electrolytes and water, which makes edemas. Lymphatic system in normal conditions takes albumins, electrolytes and water, and brings them again into the vein drainage system. In those conditions the lymphatic net is not capable to take over transported role, because a muscle pump is missing. Edema brings cascade reaction of hypoxia and tissue adaptation in new conditions. On our experimental model, in both groups during arterialisation of the vein system, the nerve was cut. Denervation in this case widens the venous vascular net and decreases vascular wall tonus. It is considered that opening of arteriovenous shunts and precapillary sphincter are more expressed when denervation action is done [18].

The experimental rabbit model with demonstration AVF by Byun et al. [19] carries out "shorted" circle of perfusion in the rabbit ear with anastomosis of afferent central artery and anterior branch of central vein without the delay method. Arterial blood perfusion in venous vascular space has no possibilities for perfusion in distal ear parts. Total flap necrosis occurs in all AVF**,** despite the previous T-T micro anastomosis. A high percentage of AVF necrosis is similar to that reported in 1997 by Cho et al. [20], in 2003 by Morhammer et al. [21] and in 2005 by Baser et al. [22], on pedicle venous flap on rats.

According to this, it was established that denervation and ischemia improve surface enlargement of a flap with the delay method performed [23–25]. There are other explanations, based on the fact that surface enlargement of flap by the delay method is directly connected to the level of denervation. Since denervation in the elevated flap is bigger, the surface and level of necrosis is smaller, and the opposite way round. Delay of AVF was for the first time demonstrated by Nakayama et al. [6] in 1981, on the epigastic skin flap model in rats. The results were confirmed in the similar study of Voukidis [7]. Despite these promising results, the performed experimental model was not appropriate for two separate microsurgical procedures; venous flap arterialisation and delay method, with significant artery manipulation on the donor site with possible morbidity.

Clinical usage of big AVFs, was not achieved due to complex tissue. The first successful AVF in 1981 had the surface of only a few cm^2. Enlargement of the surface of this flap by the delay method had a limited success. In 1995 Byun et al. [19] examined the delay method of AVF on the rabbit ear. Both ears in 25 New Zealand white rabbits (n = 50) were randomized in three experimental groups. In the first one, AVFs were performed. In the second and the third one surgical delay method was performed, marked as limited and extensive. Two weeks after arterialisation of venous flap was done as in the first group, by end-to-end anastomosis of central blood vessels. The central vein above communicant branch with anterior marginal vein was excluded from blood flow by ligation. After two weeks flap surface was compared in all groups after arterialisation of venous system. In the non delay group, first one, all flaps necrotized completely (100%). Tissue vitality was 0%. In the second group the surface of AVF was 67.9% and in the third 94%, so the surface size of AVF was significantly increased by the delay method. During analysis of previous experimental models of AVF on the rabbit ear, in which the delayed method was performed to enlarge flap surface, we concluded that designed AVF models were hemodynamically inviolable. The mentioned author excluded some parts of the venous system of the rabbit ear from circulation (in our

opinion) making better conditions for AVF survival (delayed method performed).

After a preliminary research we designed AVF simply for experimental work using the surgical delay method. In this way it was more realistic to view surface enlargement of AVF after surgical delay method. AVF survival is optimized by using smaller-caliber veins for inflow and reserving larger-caliber veins for outflow. This regulates inflow and eliminates high blood pressure, and AVF behaves as physiologic flaps do, by not relying on neovascularisation for survival [26].

Conclusion

In the first experimental animal group a venous flap was arterialised with no surgical delay method. The average flap surface was 64.50 cm^2, and the average necrosis surface 42.66 cm^2. In the second experimental animal group a flap was arterialised with the surgical delay method. The average flap surface was 94.04 cm^2, and the average necrosis surface 9.01 cm^2. This clearly points out that surgical delay method usage significantly improves AVF circulation and vitality, as well as AVF surface.

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Cerebral edema in drug addicts

Edem mozga kod zavisnika od droge

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Abstract

Background/Aim. The effect of drugs leaves permanent consequences on the brain, organic in type, followed by numerous manifestations, and it significantly affects the development of mental dysfunctions. The clinicians are often given a task to estimate a patient's personality during treatment or during experts estimate of a drug addict. The aim of this research was to determine the differences, if any, in characteristics of addicts experience and personality traits in drug addicts with or without cerebral edema. **Methods.** The research was conducted on a sample of 252 male drug addicts, the average age of 23.3 (SD = 4.3) years. Cerebral edema was confirmed on magnetic resonance (MR) images of the brain performed during the treatment of the addicts. The participants were tested by the psychologists using Minnesota Multiphasic Personality Inventory (MMPI-201) test, and the data were processed using canonical discriminate analysis within the SPSS program. The dependent variable in the study was cerebral edema. A block of independent variables, designed for the requirements of this study, consisted of two subgroups. The first one consisted of 12 variables describing the relevant characteristics of drug abuse. The second subgroup consisted of 8 psychopathological tendencies in the personality defined by the mentioned test. **Results.** Cerebral edema was confirmed in 52 (20.63%) of the drug addicts. The differences between the groups of drug addicts with and without cerebral edema were determined in the following: the time span of taking drugs (0.301), use of alcohol parallel with drugs (0.466), and treatment for addiction (0.603). In the drug addicts with a cerebral edema, MMPI-201 confirmed the increase in the scales for hypochondria, psychopathic deviations and psychastenia, and the decrease in the scales for schizophrenia and depression. **Conclusion.** Our study confirmed a possible connection between cerebral edema and personality traits in a number of the examined drug addicts. Considering the fact that practice often requires personality estimation, regardless whether it is about treatment or expert's estimate, it is necessary to further research in this direction.

Key words:
brain edema; substance-related disorders; personality disorders.

Apstrakt

Uvod/Cilj. Dejstvo droge ostavlja trajne posledice organskog tipa, na mozak, praćene brojnim neurološkim manifestacijama, i bitno utiče na razvoj psihičkih poremećaja. Kliničarima se često postavlja zadatak da procene ličnost tokom lečenja ili veštačenja zavisnika od droge. Cilj istraživanja bio je utvrđivanje karakteristika narkomanskog staža i osobina ličnosti kod zavisnika od droge sa i bez edema mozga. **Metode.** Istraživanje je sprovedeno u grupi od 252 zavisnika od droge muškog pola, prosečne starosti 23,3 (SD = 4,3) godine. Za vreme lečenja zavisnika od droge rađena je magnetna rezonanca mozga, a za procenu psihopatoloških tendencija ličnosti korišćen je *Minnesota Multiphasic Personality Inventory* (MMPI-201) test. Za obradu podataka korišćena je kanonička diskriminativna analiza u sklopu SPSS programa. Zavisna varijabla u istraživanju bio je edem mozga. Blok nezavisnih varijabli, sačinjen za potrebe ovog istraživanja, sastojao se od dve podgrupe. Prvu je činilo 12 varijabli kojima se opisuju relevantna obeležja zavisnosti od droga. Drugu je činilo 8 psihopatoloških tendencija ličnosti definisanih pomenutim testom. **Rezultati.** Edem mozga utvrđen je kod 52 (20,63%) zavisnika od droge. Razlike između grupa zavisnika od droge sa i bez edema mozga utvrđene su u dužini narkomanskog staža (0,301), korišćenju alkohola paralelno sa drogama (0,466) i lečenju zavisnosti (0,603). Kod zavisnika od droge sa cerebralnim edemom na MMPI-201 testu utvrđene su povišene skale hipohondrije, psihopatske devijacije i psihastenije, a sniženje skala šizofrenije i depresije. **Zaključak.** Kod jednog broja ispitivanih zavisnika od droge potvrđena je sumnja u mogućnost povezanosti edema mozga i osobina ličnosti. S obzirom na to da praksa često iziskuje potrebu za procenom ličnosti, bilo da se radi o lečenju ili veštačenju, neophodno je nastaviti istraživanja u ovom pravcu.

Ključne reči:
mozak, edem; poremećaji izazvani supstancama; ličnost, poremećaji.

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Introduction

Without doubt, drug addiction is accompanied by numerous social, mental and medical disorders. Nowadays, it is almost certainly known that the use of drugs is closely associated with dysfunctions in the functioning of the central nervous system (CNS) and the nervous system in general. Medical practice recognizes many conditions and diseases that could be initiated by or manifested among drug addicts. Most frequently, these manifestations include the crises of consciousness, seizures, ischemic changes in the brain, cerebral edema, polyneuropathy, various forms of metabolopathies, etc [1] .

Recently, a significant increase in the number of cerebrovascular diseases by the type of ischemia has been noticed among younger population. According to the authorities in this field, uncontrolled and excessive intake of certain medicines and drugs affect this CNS disease. Cerebral edema is a frequent comorbidity of CNS diseases. In this context, the most frequently mentioned drugs are amphetamines, cocaine, phenicyclidine, as well as natural and synthetic medications that have pharmacological effects similar to morphine – the so-called opioids [2]. Data on possible research, closely related to this work could not be found in the literature available to us.

Although cerebral edema is primarily a medical phenomenon, it is not absolutely independent on the individual physiological features. Sufficient proof of this is the fact that drug abuse has an important place in the etiology and mechanism of its formation. Long-term experience in working with drug addicts motivated the authors to research possible influence of cerebral edema on personality traits. The reason for this is that drug addicts often suffer from various neurological disorders, and that their treatment requires team work and more often than not the expertise of the committed felony. Consequently, the nature of the research calls for setting up one general and two specific hypotheses, in accordance with the groups of independent variables. The general hypothesis relates to the joint role of variables of the drug addicts' experience and personality traits as follows: There is a significant, but limited contribution of situational and personal factors to differentiating between the incidences of cerebral edema in drug addicts. The first particular hypothesis concerns the variables in drug addicts' experience: Variables that are typical of drug addicts significantly affect the differentiation of drug addicts with or without edema. The other particular hypothesis that refers to personality traits is as follows: The personality traits that bear the character of pathological tendencies have no effect on the distinguishing addicts with or without cerebral edema. Due to the mentioned, the aim of this study was to determine the existence of differences in the characteristics of addicts' experience and personality traits among drug addicts with or without cerebral edema.

Methods

The investigation was conducted from 2007 to 2012 on 252 male drug addicts, aged between 19 and 25, average 23.3 (SD = 4.3) years of age. Male addicts were selected for the purposes of the research, because, according to the experience of the authors, they outnumber female addicts in both drug abuse and committing felony, and are as such more available for the research. The patients had been hospitalized, semi-hospitalized and treated in out-patient clinics in a number of health institutions: Clinic for Psychiatry and Mental Health in Novi Sad, private institutions for treatment of addiction such as "Dr Vorobljev" Hospital in Zemun, "Lorijan" Hospital in Belgrade; "Vita" Hospital in Novi Sad; SO-VIL General Practice (GP) Office, and at the extended home treatment in Dispensaries "Novi Sad" in Novi Sad. The data were processed only for the purposes of this research in the SO-VIL GP Office in Novi Sad. During the treatment of addicts whose clinical manifestations were the basis for a reasonable doubt that they suffer from CNS ailment, magnetic resonance imaging (MRI) of the brain was required. Most frequent issues addicts complained about were a diffuse headache, varying in intensity and duration, feeling of tension in the head, occasional vertigo, nausea, tinnitus, and photophobia. The neurological examination was within the physiological limits. MRI confirmed the cerebral edema, while other CNS diseases (head injuries, anomalies and brain tumors) and similar were not confirmed. Cerebral edema was diagnosed in 52 (20.63%) of the cases.

The psychologists who professionally estimated addicts' personality used the Minnesota Multiphasic Personality Inventory (MMPI-201). The test has 11 scales of which the first 8 measure classical psychopathic personality dimensions, while the remaining 3 scales are control scales and are designed to assess the validity of the obtained materials of the test material [3, 4]. The data about the addicts' experience were collected in a specially designed questionnaire, thought up based on the experience of the researchers, and adapted to the needs of this research. The dependent variable is a binary-encoded data about cerebral edema. It is a category variable and that is why the analysis of the difference among subjects who are and are not diagnosed with cerebral edema is done using a canonical discriminate analysis. The independent variables comprise two groups of dimensions contained in the previously mentioned questionnaire. The first group that describes the addict's experience is marked with factors that define the shape of the addiction of the given individual. They are: age when first using drugs; length of drug use (in months); frequency of drug use; the amount of drugs used daily; using alcohol along with the drugs; using tablets along with drugs; using cocaine; venous drug application; whether he or she was treated for addiction; length of abstaining periods; using blockers of opiate receptors; using methadone. The first 4 variables are numerical and the others are categorical. Each category is especially binary coded (and thus became a new variable) to be available for the foreseen model of data analysis [5].

The second group is related to the personality traits of psychopathological connate space. The instrument that was used in the process of psychological expertise (MMPI-201) is approximated by the following 8 properties: hypochondria, depression, hysteria, psychopathic deviation, paranoia, psychastenia, schizophrenia, hypomania, while the function of

the last 3 dimensions: defense mechanism, rigidity and confused thinking is to check the validity of the protocol.

All the properties have the form of interval scales. The result of the subjects at subtests that measure these dimensions is expressed as a total score at each of the subscales.

For the research into the difference among the forms of cerebral edema of drug addicts within the variables of addicts' experience and personality traits, the canonic discriminate analysis was used within the SPSS programmed for statistical processing of data.

Results

The analysis of differences in the addicts experience and personality traits between the drug addicts who have or do not have a diagnosed cerebral edema begins with a matrix of univariate tests of equality of arithmetic means of the group of addicts with or without cerebral edema at each independent variable as shown in Table 1.

these differences suggest that there will be a total discrimination between the two groups.

According to the results obtained in this paper for the existence of differences in the diagnose of cerebral edema in the drug addicts, in the space that is defined by some variables of their experience and pathological tendencies in their personality, one canonical dimension is responsible (λ = 0.269, Wilks lambda 0.788, χ^2 = 56,810, $p < 0.000$), the canonical correlation which was 0.460. This dimension could explain 21.16% of the variance of the existing differences.

The meaning of that discriminate function is determined by standardized canonical discriminate coefficients of independent variables, and the orthogonal projections of these variables on the discriminate function. In the first case, it is about the so-called regression ponders, and in the other about the correlations of the variables with the function, i.e. about the structure of the discriminate function. These results are given in Table 3.

Table 1

Characteristics of experience among the addicts with/without cerebral edema[0]

Caracteristics of addicts	Wilks' lambda	F	Significance
Age of the first using drugs	0.978	5.519	0.020
Length of drug use	0.929	19.195	0.000
Frequency of drug use	0.983	4.359	0.038
The amount of drugs used daily	0.956	11.384	0.001
Using alcohol along with the drugs	0.975	6.504	0.011
Using tablets along with drugs	1.000	0.077	0.781
Using cocaine	1.000	0.068	0.795
Intravenous drug administration	0.962	9.950	0.002
Treatment for addiction	0.999	0.127	0.722
Length of abstinence	0.975	6.479	0.012
Using blockers	0.992	2.046	0.154
Using methadone	0.994	1.481	0.225

Table 2

Characteristics of personality traits among addicts with/without cerebral edema[0]

Characteristics of personality traits	Wilks' lambda	F	Significance
Hypochondria	0.995	1.999	0.275
Depression	0.995	1.265	0.262
Hysteria	1.000	0.001	0.977
Psychopathic deviation	0.992	2.043	0.154
Paranoia	0.999	0.226	0.635
Psychastenia	1.000	0.071	0.790
Schizophrenia	0.989	2.880	0.091
Hypomania	0.999	0.249	0.618
Defense mechanism	1.000	0.003	0.953
Rigidity	0.999	0.300	0.584
Confused thinking	0.994	1.465	0.227

The matrix univariate tests of equality contains a summarized one-way analysis of variance for each variable. Univariate tests are not identical to the actual F-tests, because they are derived from the existing discriminatory solution and do not have a particular significance because of this and because derivativeness of univariate quality, apart from indicating that with some variables there were significant differences in the average values between the groups and that

Judging from the coefficients obtained the substantial contribution to the function of separating addicts with and without cerebral edema are the variables that define the addicts experience, while the results obtained through variables analysis which describe psychopathological tendencies of personality have significantly lesser predictive power apart from hypochondria, depression, psychastenia (Table 3).

Table 3

Canonical discriminate function of characteristics in the group of addicts with/without cerebral edema[0], their experience and personality traits

Variables	Discrim. coefficient	Structure of function
Age of the first using drugs	-0.277	-0.286
Length of drug use (in months)	0.301	0.534
Frequency of drug use	-0.042	0.255
The amount of drugs used daily	0.241	0.411
Using alcohol along with the drugs	0.466	0.311
Using tablets along with drugs	-0.087	-0.034
Using cocaine	0.152	0.385
Intravenous drug administration	-0.269	0.032
Treatment for addiction	0.603	-0.044
Length of abstinence	0.297	0.310
Using blockers	-0.698	-0.175
Using methadone	-0.394	-0.148
Hypochondria	-0.493	-0.134
Depression	0.455	0.137
Hysteria	0.093	-0.004
Psychopathic deviation	-0.223	0.174
Paranoia	-0.025	0.058
Psychastenia	-0.359	-0.033
Schizophrenia	0.542	0.207
Hypomania	-0.075	-0.061
Defense Mechanism	0.203	0.007
Rigidity	-0.024	-0.067
Confused Thinking	0.019	0.148

Based on the prognostic power of an isolated function, centroids of the groups on the discriminate function and the results of the classification of addicts into the two groups speak of the nature and the role of influences of the drug addicts experience variables and pathological tendencies in the personality on differentiating the addicts with and without cerebral edema. The data on the group centroids show that there is a clear difference in the experience of addicts and pathological tendencies in personality between addicts groups with cerebral edema (1.013) and without cerebral edema (-0.263). The predicted affiliation was confirmed with 74.6% of successfully grouped drug addicts. As far as the results of the classification made on the basis of discriminate functions, there is a satisfactory level of agreement between both procedures. The success of this method lies in the fact that, based on the isolated latent dimension and utilized sample of independent variables, one can recognize the real situation and place the vast majority of addicts in groups, where they belong, based on the objective diagnosis.

Discussion

The results unambiguously confirm that certain variables of the addicts experience and pathological tendencies of personality have significant canonical discriminate coefficients with a function responsible for the differentiation of drug users with and without a diagnosed cerebral edema.

In the case of variables used to analyze the drug experience, we confirmed that the use of drugs has an impact on the occurrence of edema due to biochemical processes during intoxication of the nervous system. It is therefore logically that the drug as an external stimulus appears as an outer risk factor. This study suggests that it involves a small part of that variance, not only because the canonical correlation of the discriminate function is relatively low, but also because the drugs are only one of the conditions that are to be met for the occurrence of edema.

What is surprising is the direction of the impact of some of these variables on the creation of these differences. Judging by the signs of canonical coefficients (and correlations), and bearing in mind that the input data are numerated, these unexpected results indicate that the cerebral edema will be more frequently found in drug addicts who started using drugs later (aged 18–20) rather than earlier in life (aged 13–18), and who have spent less time in drug use and take smaller amounts of drugs on a daily basis. A question arises as to whether a complete maturation of the brain after 18 years of age, is also a better ground for the development of cerebral edema, rather than a younger age, when the development of the brain is not yet complete. What is also surprising is that using pills (sedatives, psychostimulants, analgesics, antipsychotics, etc.) along with drugs played no role in the differences of the variables formation. Considering the particularity of the relations that we analyzed, in quotes from literature available to us and in the journals that deal with this issue, we found no data that could be compared with our results. The direction of the impact of other variables of the addicts experience is expected and it is easy to understand: the use of alcohol along with drugs, cocaine use, lack of treatment and lack of abstinence increase the likelihood of cerebral edema and, consequently, its effect on the characteristics and behavior of addicts [6–8].

When the pathological tendencies in the person are concerned, the direction of defining the functions (discriminate coefficients) shows that high hypochondria, psychopathic deviations and psychastenia, and low depression and schizophrenia confirm the differences in the existence of cerebral edema. Hysteria, paranoia, hipomania, rigidity and

lenience to confused thinking had zero impact on those differences. The mentioned claims point to the existence of interconnectedness of the examined relation. Given the clinical importance and the nature of the independent variables, the scope of 21.16% variance difference, is under no circumstances to be underestimated. For these reasons, the value of the MMPI test scales could contribute to the quality of the experts findings.

Some psychologists do not even recommend the use of the MMPI test (and no other, for that matter) in the case of suspected or proven organicism of any origin even with addiction to drugs. A number of psychologists do not support those claims and in case of need for psychological processing they regularly use this test. The possibility that these results are related to the existence of cerebral edema is certain, because the addict is tested during the treatment or within pre-existing clinical images. Nevertheless, we are confident that the scores were not significantly influenced by these changes and that they reflect the real picture of a personality. For this, there is some important empirical evidence. Firstly, the correlations of some of these properties to a variable 'cerebral edema' are not statistically significant or are very low (paranoid 0.35, psychastenia 0.34, confusing thinking 0.32).* If these properties depended on the cerebral edema there would be correlations with all of them even at the lowest substantial level. Secondly, there are significant differences in arithmetic means of each of these traits among addicts with and without cerebral edema. These differences would have to have existed if cerebral edema had impact on these properties. Thirdly, there are no significant differences in the arithmetic means in any characteristics between the subjects who were addicted to drugs and those who are not [4, 6] This result refutes even the argument that addiction itself changes the structure of personality traits and affect the test results. Unfortunately, we found no results of this relation in the literature available to us, and we could not make comparison.

With this in mind we can claim with certainty that the results of the trend in the personality, which the MMPI covers, are valid. This means that to a significant degree high hypochondria, psychopathic deviation, psychastenia, and low depression and schizophrenia allow predicting of the differences in the presence of cerebral edema between the two groups of drug users. Variables of addicts experience have the impact to the same degree. We realize that psychopathic tendencies in personalities are predispositions of an individual and characteristics of their personality set, and that the symptoms of organicism are due to the impact of drugs on the brain [1]. The importance of the results is not only of practical but is also of theoretical importance. It refers to the observation that based on personality traits one can predict the existence of changes in the central nervous system. This study shows that there are relationships between mental reality, and its physiological basis, which are mathematically provable, outside laboratories in simple paper-pencil situations.

Cerebral edema was diagnosed in 52 (26.63%) of the cases, which corresponds to the obtained value of the predicted affiliation of 74.6% of the total of the studied drug addicts. The obtained value of the canonical discriminative analysis points to the fact that one canonical dimension, whose canonical correlation of 0.460 is responsible for the existence of the tested relation. This dimension can explain 21.16% of variance in the existing differences.

Conclusion

The direction towards which function defining (of discriminative coefficients) of the observed psychopathological personality tendencies is going shows that high hypochondria, psychopatic deviation and psychastenia, and low depression and schizophrenia confirm the differences regarding the existence of cerebral edema. The results obtained in this research point to a high importance of the examined relation and also to the fact that Minnesota Multiphasic Personality Inventory test should find an important place in diagnosing and treating drug addicts as well as in experts estimation work. For these reasons, it is important to continue research in this direction.

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*Two of these three features are irrelevant, since only psycastenia solely has influence on a discriminant function.

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Bone turnover markers in medicamentous and physiological hyperprolactinemia in female rats

Markeri koštanog metabolizma u medikamentnoj i fiziološkoj hiperprolaktinemiji kod ženki pacova

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Abstract

Background/Aim. There is a lack of data on the effects of prolactin on calcium metabolism and bone turnover in hyperprolactinemia of various origins. The aim of this study was to compare the influence of medicamentous and physiological hyperprolactinemia on bone turnover in female rats. **Methods.** Experimental animals (18 weeks old, Wistar female rats) were divided as follows: the group P – 9 rats, 3 weeks pregnant; the group M3–10 rats that were intramuscularly administrated sulpirid (10 mg/kg) twice daily for 3 weeks, the group M6 – 10 rats that were intramuscularly administrated with sulpirid (10 mg/kg) twice daily for 6 weeks, and age matched nulliparous rats as the control group: 10 rats, 18-week-old (C1) and 7 rats, 24 weeks old (C2). Laboratory investigations included serum ionized calcium and phosphorus, urinary calcium and phosphorous excretion, osteocalcin and serum procollagen type 1 N-terminal propeptide (P1NP). **Results.** Experimental animals in the group P compared to the control group, displayed lower mean serum ionized calcium (0.5 ± 0.2 *vs* 1.12 ± 0.04 mmol/L; $p < 0.001$); higher mean serum phosphorus (2.42 ± 0.46 *vs* 2.05 ± 0.2 mmol/L; $p < 0.05$); increased urinary calcium (3.90 ± 0.46 *vs* 3.05 ± 0.58; $p < 0.01$) and significantly increased P1NP (489,22 ± 46,77 *vs* 361.9 ± 53,01 pg/mL; $p < 0.001$). Experimental animals in the group M3 had significantly decreased P1NP, compared to the contol group. Prolongated medicamentous hyperprolactinemia (the group M6) induced increased serum ionized calcium (1.21 ± 0.03 *vs* 1.15 ± 0.02 mmol/L; $p < 0.001$); decreased serum phosphorus (1.70 ± 0.13 *vs* 1.89 ± 0.32 mmol/L; $p < 0.001$); decreased osteocalcin and P1NP. **Conclusions.** Physiological hyperprolactinemia does not have such harmful effect on bone metabolism as medicamentous hyperprolactinemia. Chronic medicamentous hyperprolactinemia produces lower serum levels of bone formation markers. Assessment of bone turnover markers in prolongated medicamentous hyperprolactinemia provides an opportunity for earlier diagnosis of bone metabolism disturbances and should be considered as mandatory.

Key words:
hyperprolactinemia; pregnancy; sulpiride; rats; osteogenesis; biological markers; calcium; phosphorus; osteocalcin.

Apstrakt

Uvod/Cilj. Nema dovoljno podataka o efektima prolaktina na metabolizam kalcijuma i koštani promet kod hiperprolaktinemije različitog porekla. Cilj ovog rada bio je da uporedi uticaj medikamentne i fiziološke hiperprolaktinemije na koštani metabolizam kod ženki pacova. **Metode.** Eksperimentalne životinje (ženke pacova soja Wistar, stare 18 nedelja) podeljene su na sledeće grupe: grupa P – devet pacova u trećoj nedelji trudnoće; grupa M3 – 10 pacova, kojima je tokom tri nedelje, dva puta dnevno, davan intramuskularno sulpirid (10 mg/kg); grupa M6 – 10 pacova kojima je tokom šest nedelja, davan sulpirid (10 mg/kg) intramuskularno, dva puta dnevno; starosno odgovarajuće nulipare ženke pacova kao kontrolne grupe: C1 – 10 pacova, starih 18 nedelja i C2 – sedam pacova, starih 24 nedelje. Određivana je koncentracija jonizovanog kalcijuma i fosfora u serumu, 24-časovno izlučivanje kalcijuma i fosfora urinom, osteokalcin i serumski amino terminalni propeptid prokolagena tipa I (P1NP). **Rezultati.** U poređenju sa kontrolnom grupom eksperimentalne životinje u grupi P imale su snižen jonizovan kalcijum u serumu (0,5 ± 0,2 *vs* 1,12 ± 0,04 mmol/L; $p < 0,001$), povišene fosfate u serumu (2,42 ± 0,46 *vs* 2,05 ± 0,2mmol/L; $p < 0,05$), povećano 24-časovno izlučivanje kalcijuma urinom (3,90 ± 0,46 *vs* 3,05 ± 0,58 mmol/24 h; $p < 0,01$) i značajno povišen P1NP (489,22 ± 46,77 *vs* 361,9 ± 53,01 pg/mL; $p < 0,001$). Značajan pad P1NP zabeležen je u eksperimentalnoj grupi M3 u poređenju sa kontrolnom grupom. Prolongirana medikamentna hiperprolaktinemija u grupi M6 dovela je do porasta jonizovanog kalcijuma u serumu (1,21 ± 0,03 *vs* 1,15 ± 0,02mmol/L; $p < 0,001$), pada fosfata (1,70 ± 0,13 *vs*

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1,89 ± 0,32 mmol/L; $p < 0,001$) i sniženja koncentracije osteokalcina i P1NP. **Zaključak.** Fiziološka hiperprolaktinemija u manjoj meri utiče na koštani metabolizam nego medikamentna hiperprolaktinemija. Hronična medikamentna hiperprolaktinemija dovodi do pada koncentracije P1NP, što je odraz snižene koštane formacije. Rutinsko određivanje biohemijskih markera koštanog metabolizma u prolongiranoj medikamentnoj hiperprolaktinemiji pruža mogućnost ranije dijagnoze poremećaja koštanog metabolizma.

Ključne reči:
hiperprolaktinemije; trudnoća; sulpirid; pacovi; osteogeneza; biološki pokazatelji; kalcijum; fosfor; osteokalcin.

Introduction

Hyperprolactinemia (HP) is a common hypothalamic-pituitary axis disorder. This "abnormal laboratory value" may be caused by any process interfering with dopamine synthesis, its transport to the pituitary gland or its action on lactotroph dopamine receptors [1]. Considering the complexity of various etiologies, HP could be divided into 4 categories: physiological, pathological, medicamentous and functional HP.

The most frequent clinical symptoms of HP, regardless of its origin, are galactorrhea, oligo- or amenorrhea and sterility in women and impotence, libido loss and gynecomastia in men. In last few decades, there is growing evidence of decreased bone mineral density (BMD) and increased activity of bone turnover markers caused by HP. Prolactin (PRL)-secreting pituitary tumors in people [2–6] and rats [7] are associated with osteopenia. Antipsychotic-induced HP can cause osteoporosis and increased risk of hip fracture [8–10]. Pregnancy and prolonged lactation, conditions with physiological HP, can lead to a significant bone loss [11–13]. Although rapid mineralizing neonatal skeleton (during pregnancy) and higher calcium demand for milk production (during lactation) places significant stress on maternal calcium homeostasis, bone loss is usually recovered after weaning [14–16]. Longidutinal studies have shown that there is no detrimental effect of parity and prolonged breast-feeding on long-term bone health [17, 18].

Although physiological and medicamentous HP have different final effects on skeletal system, there is a lack of data regarding the effects of PRL on calcium metabolism and bone turnover in HP of various origins.

The aim of this experimental study was: 1) to determine if there was a difference in calcium metabolism during pregnancy (physiological HP) and in sulpirid-induced HP (medicamentous HP); 2) to compare the influence of medicamentous and physiological HP on bone turnover markers; 3) and to reveal a possible effect of prolonged medicamentous HP on calcium metabolism and bone turnover markers.

Methods

Animals

Pregnant and age matched nulliparous Wistar female rats (18 weeks old) were obtained from the Animal Laboratory Centre Torlak, Institute for Medical Research, Military Medical Academy, Belgrade, Serbia. Experimental study was conducted in Biomedical Research Center, Medical Faculty, University of Niš, Serbia. The weight of experimental animals ranged 290–340 g. They were housed under a 12 : 12 h light-dark cycle (lights on at 06 h) and fed standard chow and water. Room temperature was 23–25°C with average humidity of 50–60%. The study was approved by the Ethical Committee of the Medical Faculty, University of Niš, Serbia.

Experimental design

Experimental animals were divided into the following groups: 9 rats, 3 week pregnant (P – physiological HP during pregnancy; gestation period in Wistar rats 19–22 days); 10 rats with intramuscularly administrated sulpirid (10 mg/kg) twice daily for 3 weeks (M3 – medicamentous HP); 10 rats with intramuscularly administrated sulpirid (10 mg/kg) twice daily for 6 weeks (M6 – medicamentous HP). Since bone growth and calcium accretion are normally age dependent, we used age matched nulliparous rats as control groups: 10 rats, 18 weeks old (C1), and 7 rats, 24 weeks old (C2). All rats in each group (pregnant, medicamentous treated and controls) were sacrificed on the same day.

Laboratory investigations

In order to confirm HP serum PRL levels were measured in all experimental groups and compared with controls. PRL concentration was measured using enzyme-linked immunosorbent assay kit for PRL. The kit is a sandwich enzyme immunoassay for *in vitro* quantitative measurement of PRL in rat serum, plasma and other biological fluids (manufactured by Uscnk, Life Science Inc.).

All experimental animals were analyzed for serum ionized calcium and urinary calcium, inorganic phosphorus and urinary phosphate. All rats, in each group, were kept in single rat metabolic cages, 24 h before they were sacrificed, in order to collect 24 h urine for calciuresis and phosphorus diuresis. Rats were anesthetized with intramuscular injection of 10% ketamine hydrochloride (0.3 mL *per* animal). Blood samples untill exsanguination were taken by punction of left myocardial ventricle through midline thoracoabdominal incision. Mineral assays were done by the following methods: serum ionized calcium by potentiometric method; urine calcium by photometric colour test (Beckman Coulter, OLYMPUS analyzer); serum and urine phosphate concentration by photometric UV test (Beckman Coulter, OLYMPUS analyzer); The bone turnover markers studied were: osteocalcin (OC) and serum procollagen type 1 N-terminal propeptide (P1NP). The methods used for bone turnover markers were: OC by electrochemiluminescence immunoassay (N-MID Osteocalcin, Cobas, Roche) and P1NP was measured using enzyme-linked immunosorbent assay kit for P1NP (Uscnk, Life Science Inc.).

Statistical analysis

Data were analyzed using SPSS (version 15.0). Continuous (measurable) parameters were presented with mean values ($\bar{x}$) and standard deviation (SD), median (md), maximum (max) and minimum (min) values. The Shapiro-Wilk test was used to determine normality of parameters distribution. Differences were tested by Student's *t*-test for independent samples if the distribution of parameters was normal and Mann-Whitney *U*-test was used if parameters distribution was deviated. We used Student's *t*-test for dependent samples (normal distribution) and Wilcoxon test (deviated distribution) to test statistical significance between continuous parameter values at the beginning and the end of the study.

Results

Changes in prolactin concentration, calcium metabolism and bone turnover markers in physiological hyperprolactinemia

PRL concentrations were significantly higher during the third week of pregnancy (P), compared with C1 (181.80 ± 29.65 *vs* 105.38 ± 28.34 pg/mL; $p < 0.001$) (Table 1).

OC concentrations were significantly lower during pregnancy compared to C1 (9.01 ± 1.09 ng/mL *vs* 17.50 ± 2.76 ng/mL, $p < 0.001$) (Table 1).

P1NP concentrations were significantly higher in physiological HP (P) in comparison to that in the C1 group (489.22 ± 46.77 pg/mL *vs* 361 ± 53.01 pg/mL, $p < 0.001$) (Table 1).

Serum ionized calcium concentrations were significantly decreased in pregnancy (0.5 ± 0.2 mmol/L *vs* 1.12 ± 0.04 mmol/L, $p < 0.001$) followed by significantly increased phosphorus compared to C1 (2.42 ± 0.46 mmol/L *vs* 2.05 ± 0.19 mmol/L; $p < 0.05$) (Table 2).

Urinary calcium and phosphorus excretion (measured as daily total calcium and daily phosphorus excretion) significantly increased during pregnancy compared to the control group (urinary calcium 3.90 ± 0.46 mmoL/24 h *vs* 3.05 ± 0.58 mmoL/24 h; $p < 0.01$; urinary phosphorus 141.15 ± 20.65 mmoL/24 h *vs* 45.54 ± 7.99 mmoL/24 h, $p < 0.001$) (Table 2).

Changes in prolactin concentration, calcium metabolism and bone turnover markers in medicamentous hyperprolactinemia

Significantly increased PRL levels in sulpirid-treated rats, compared to age matched controls, confirmed the state of medicamentous HP (M3: 182.03 ± 57.80 *vs* 105.38 ± 28.34 pg/mL; $p < 0.001$; M6: 148.92 ± 20.46 *vs* 112.01 ± 11.92 pg/mL, $p < 0.001$). Even though lower PRL concentration were verified in M6 in comparison with M3, decrease was not significant (148.92 ± 20.46 *vs* 182.03 ± 57.80 pg/mL, $p > 0.05$).

Table 1

Concentration of prolactin, osteocalcin and P1NP in experimental groups

Experimental groups	Prolactin (pg/mL) $\bar{x}$ ± SD	Osteocalcin (ng/mL) $\bar{x}$ ± SD	P1NP $\bar{x}$ ± SD
C_1	105.38 ± 28.34	17.5 ± 2.76	361.90 ± 53.01
P	181.8 ± 29.65	9.01 ± 1.09	489.22 ± 46.77
M_3	182.03 ± 57.8	15.28 ± 2.51	309.60 ± 36.74
M_6	148.92 ± 20.46	13.55 ± 3.42	291.70 ± 71.03
C_2	112.01 ± 11.92	16.18 ± 2.0	314.86 ± 50.99
p	< 0.001 (P : C_1) < 0.001 (M_3 : C_1) < 0.001 (M6 : C_2)	< 0.001 (P : C_1)	< 0.05 (M_3 : C_1) < 0.001 (M_3 : P)

Experimental groups: P – physiological hyperprolactinemia (HP) during pregnancy; M3 – medicamentous HP with a 3-week duration; M6 – medicamentous HP with a 6-week duration; C1 – control group age matched with P and M3; C2 – control group age matched with M6; $\bar{x}$ ± SD – mean value; SD – standard deviation.

In medicamentous HP (M3), serum calcium levels were higher, with no significant difference compared to C1 (1.15 ± 0.04 *vs* 1.12 ± 0.04 mmol/L) but serum calcium levels in physiological HP were significantly decreased in comparison with M3 (0.5 ± 0.2 *vs* 1.15 ± 0.04 mmol/L, $p < 0.001$). Phosphorus concentrations were not significantly changed during 3 weeks of medicamentous HP compared to C1 (2.14 ± 0.48 *vs* 2.05 ± 0.19 mmol/L, $p > 0.05$). Even though lower phosphorus concentration were verified in comparison with pregnant rats, a decrease was not significant (2.14 ± 0.48 *vs* 2.42 ± 0.46

Table 2

Calcium and posphorous serum concentrations and 24-h urine exeretion values

Parameter	C1 (n = 10)	P (n = 9)	M3 (n = 10)	M6 (n = 10)	C2 (n = 7)
Serum ionized calcium (mmol/L), $\bar{x}$ ± SD	1.12 ± 0.04	0.50 ± 0.20‡	1.15 ± 0.04	1.21 ± 0.03‡	1.15 ± 0.02
Serum phosphorous (mmol/L), $\bar{x}$ ± SD	2.05 ± 0.19	2.42 ± 0.46*	2.14 ± 0.48	1.70 ± 0.13	1.89 ± 0.32
Urine calcium excretion (mmol/24 h), $\bar{x}$ ± SD	3.05 ± 0.58	3.90 ± 0.46†	4.31 ± 1.11†	2.88 ± 0.60	3.37 ± 0.87
Urine phosphorous (mmol/24 h) excretion ($\bar{x}$ ± SD)	45.54 ± 7.99	141.15 ± 20.65‡	50.58 ± 9.77	53.93 ± 14.05	55.03 ± 20.37

***p < 0.05 (P *vs* C1); †p < 0.01 (P *vs* C1; M3 *vs* C1, M6); ‡p < 0.001**
P, M3, M6, C1, C2 (for explantation see under Table 1).

mmol/L). With prolongation of medicamentous HP (M6) calcium concentrations continued to rise and serum phosphorus levels to fall. After 6 weeks of sulpirid provoked HP, serum calcium concentrations were significantly increased in comparison with C2 (1.21 ± 0.03 mmol/L *vs* 1.15 ± 0.02 mmol/L, $p < 0.001$). There was no significant difference in serum phosphorus concentrations with a prolongation of sulpirid treatment (1.70 ± 0.13 mmol/L *vs* 1.89 ± 0.32 mmol/L, $p > 0.05$).

In M3 rats calciuresis was significantly increased compared to the control group (4.31 ± 1.11 mmoL/24 h *vs* 3.05 ± 0.58 mmoL/24 h; $p < 0.01$) while phosphorus diuresis was not significantly changed (50.58 ± 9.77 mmoL/24 h *vs* 45.54 ± 7.99 mmoL/24 h, $p > 0.05$).

With longer duration of medicamentous HP (M6) there were no significant changes in urinary calcium and phosphorus excretion compared to C2 (calciuresis: 2.88 ± 0.6 mmoL/24 h *vs* 3.37 ± 0.87 mmoL/24 h; phosphorus diuresis: 53.93 ± 14.05 mmoL/24 h *vs* 55.03 ± 20.37 mmoL/24 h).

OC concentrations were decreased in sulpirid-treated rats compared to age matched control groups (M3: 15.28 ± 2.51 ng/mL *vs* 17.50 ± 2.76 ng/mL; M6: 13.55 ± 3.42 ng/mL *vs* 16.18 ± 2.0 ng/mL) but without statistical significancy.

P1NP concentration in M3 compared to C1 was significantly decreased (309.60 pg/mL ± 36.74 *vs* 361.90 ± 53.01 pg/mL, $p < 0.05$) and even more in comparison with physiological HP (309.60 ± 36.74 pg/mL *vs* 489.22 ± 46.77 pg/mL; $p < 0.001$). Although the tendency of further P1NP decrease was noticed with longer duration of medicamentous HP, no significant difference was verified (291.70 ± 71.03 pg/mL, *vs* 314.86 ± 50.99 pg/mL, $p > 0.05$).

Discussion

Our study results confirmed the expected PRL increase during pregnancy. The results of mineral analyses and bone turnover markers conducted in this experimental group could be considered as representative for physiological HP.

Calcium homeostasis during pregnancy is changed due to elevated fetus demand for calcium and maternal adaptations. Adaptation mechanisms include increase in intestinal calcium absorption, decrease in urinary calcium excretion or mobilization of maternal bone mineral.

A decrease in total serum calcium concentration during pregnancy has already been reported [19, 20] and usually considered as a consequence of hemodilution and decreased serum albumin [19, 21, 22]. In order to avoid low calcium concentration due to dilutional effect, we measured serum ionized calcium level. Our study results confirmed a significant decrease in ionized calcium, which is in the contrast with previously reported results of unchanged ionized calcium throughout gestation [23, 24] and more consistent with data from several animal models, reporting fall of ionized calcium in late pregnancy [25, 26]. Rapid fetus growing in late pregnancy may exceed the maternal capacity to maintain a normal serum calcium level and result in decreased ionized calcium.

Inorganic phosphorus is very often considered to be a passive companion of calcium fluxes. Studies which evaluate phosphate balance during physiological HP (as pregnancy) are less common than calcium studies. Serum phosphate levels are usually reported as normal throughout pregnancy in humans and animals [19, 21]. Our study results showed significantly increased serum phosphorus during pregnancy. It is consistent with decreased serum ionized calcium in our experimental study, increased parathyroid hormone during rat pregnancy, reported in previous animal models [21] and a fact that dietary phosphorus is absorbed almost twice as efficiently as dietary calcium [27].

The increase of urinary calcium excretion during pregnancy is consistent with previous reports [19, 20]. It is considered as a consequence of increased calcium absorption and elevation in glomerular filtration rate (GFR) during pregnancy, which together exceed the reabsorptive capacity of the kidney [19–21].

Changes in urinary phosphorus excretion, during pregnancy, could be also due to increased dietary intake in late pregnancy, increased absorption and increased GFR during pregnancy.

OC fulfils all three of the following criteria for reliable bone turnover marker: it is osteoblast-produced protein, its increase correlates with increased bone formation, and it has fast response to changes in skeletal homeostasis. A decline in serum OC during pregnancy, in this study is consistent with the findings of previous reports [19, 20, 28, 29]. Decreased OC in pregnancy may be related to hemodilution, fetal contribution [20], increased renal degradation secondary to increased GFR [29] or lacking of normal values during pregnancy [22].

P1NP together with carboxy terminal propeptide (P1CP) are a part of the process in which type I procollagen is transformed into type I collagen. Since type I collagen constitutes 90% of bone proteins, it may be considered as very valuable and precise marker of bone formation. Our study results are consistent with limited, previously reported data, showing low P1CP and P1NP concentration in the first trimester [28, 30] with the tendency to rise above normal in the late pregnancy [30, 31]. Higher osteoblastic activity, in physiological HP, could explain faster recovery of bone loss after pregnancy and lactation.

Increased PRL levels in sulpirid-treated rats were confirmed in our study. Therefore, the results of mineral analyses and bone turnover markers, conducted in these experimental groups, could be considered as representative for medicamentous HP. With sulpirid treatment prolongation slightly decreased PRL concentrations were verified. Data from the literature usually cover the issue of different sulpiride effects, according to the low or high dosages [32]. Lower concentration of dopamine antagonist (sulpiride) can block presynaptic dopamine (D) 2 receptors, leading to decreased dopamine synthesis and release. Lactotrophs are released of dopamine inhibition and hyperprolactinemia occurs. Higher sulpiride concentrations are needed to block postsynaptic D2 receptors [33]. There are no literature data showing different effect of sulpiride with longer treatment duration. A possible explanation for decreased PRL concentration with prolongation of sulpiride treatment could be up-regulation of D2 receptors in lactotrophs after longer blocking sulpiride effect or postreceptors downstream of cAMP/calcium signalling which is necessary for PRL release [34].

Studies conducted to reveal a connection between medicamentous HP and skeletal system are often based on parameters of bone mineral density and biochemical turnover markers. There are less available data about HP influence on calcium and phosphorus levels. Our study results, showing no significant changes in serum ionized calcium and phosphorus, during a 3-week sulpirid-provoked HP, are consistent with limited previously reported data [35–37]. Even though there are growing evidences that prolonged medicamentous HP can lead to decreased bone mineral density [8–10, 36], there are still missing data about calcium and phosphorus changes during those conditions. Our study results revealed a significant calcium increase and phosphorus decrease during longer medicamentous HP. Hypercalcemia in prolonged medicamentous HP could be a result of increased calcium absorption in upper intestine (absorptive hypercalcemia), increased net bone resorption (remodelling hypercalcemia) or increased tubular calcium reabsorption (tubular reabsorptive hypercalcemia). In last decade, many experimental studies confirmed very important and direct PRL role in regulating intestinal calcium absorption [38–41]. All of these studies are based on physiological HP. There are no literature data showing that medicamentous HP also leads to increased intestinal calcium absorption. The findings of PRL receptor mRNA expression in osteosarcoma cell lines [42], cultured calvaria osteoblasts [43] and in tibia, femur and vertebrae in normal adult rats [44] suggested bones as possible direct targets of PRL. It is still uncertain whether hypercalcemia in prolonged medicamentous HP could be considered as a consequence of direct PRL influence on bones.

Renal tubular dysfunction resulting in excess calcium loss, caused by sulpirid is not so far reported. A significant fall of urinary calcium excretion in prolonged medicamentous HP was not previously reported, to our knowledge, and could be a result of some still unknown mechanisms, switched on to prevent further calcium loss.

Different studies conducted in women with major depressive disorder, with or without borderline personality disorder, before psychotropic medication, or treated with antidepressant, found increased OC [35, 45, 46]. Our study results, presenting lower OC, but still in normal referent rang, are more consistent with data provided in schizophrenic patients with antipsychotic treatment [36, 47].

There are no previously reported data, to our knowledge, about changes in P1NP in medicamentous HP. Our results, for the first time show statistical significant decrease of this osteoblastic marker in sulpirid-induced HP. Prolonged medicamentous HP leads to further fall of P1NP, reflecting poor osteoblastic activity.

Even though OC and P1NP are bone formation markers their serum levels reflect different aspects of osteoblastic activity. Osteocalcin is mostly produced during the mineralization phase, while procollagen peptides are mostly produced by proliferating osteoblasts [47].

Limitation of this study is a lack of biochemical markers of bone resorption. Comparison of bone resorption/formation rates could also be very important data about bone remodelling in physiological and medicamentous hyperprolactinemia.

Conclusion

We demonstrated herein that ionized calcium concentrations were significantly different in physiological and medicamentous hyperprolactinemia (decreased in late pregnancy and increased in sulpirid-induced hyperprolactinemia). Quite opposite influence of physiological and medicamentous hyperprolactinemia on bone formation marker procollagen type 1 N-terminal propeptide, revealed an increased osteoblastic activity in pregnancy and decreased bone formation in sulpirid-provoked hyperprolactinemia. These results provide a possible explanation why pregnancy does not determine such harmful effect on bone metabolism, while medicamentous hyperprolactinemia leads to decreased bone mineral density. The present experimental data of further procollagen type 1 N-terminal propeptide decrease in prolonged medicamentous hyperprolactinemia, provide information on dynamic, time-dependent and origin-dependent osteoregulatory roles of prolactin.

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Psychopathology and resilience in relation to abuse in childhood among youth first referred to the psychiatrist

Povezanost psihopatologije i rezilijentnosti sa zlostavljanjem u detinjstvu kod mladih upućenih na prvi psihijatrijski pregled

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Abstract

Background/Aim. Child abuse may be related to adverse psychological outcomes in adult life. However, little is known about specific clinical, family and resilience profiles of adolescents that have experienced child abuse. The aim of this study was to investigate clinical symptoms, family functioning and resilience characteristics of adolescents with the experience of abuse, first referred to psychiatrists. **Methods.** The study included 84 young participants (mean age 14.90 ± 3.10, ranging from 11 to 18 years) as consecutive first referrals to the Clinic for Children and Youth of the Institute of Mental Health, Belgrade, Serbia. The sample consisted of two groups, based on the Child Abuse Matrices of Risks. The first group included adolescents with the experience of abuse in childhood (n = 38, 13 males, 25 females), whereas the second, control group, comprised of non-abused adolescents (n = 47, 20 males, 27 females). The presence of abuse was evaluated by the Child Abuse Matrices of Risks. The study used the following questionnaires: Youth Self-Report (YSR), Adolescent Resilience Attitudes Scale (ARAS), and Self-Report Family Inventory (SFI). **Results.** Significant differences were found only among females. According to YSR, the abused girls had significantly higher scores on the Delinquent Behavior scale and marginally higher scores on Anxious/Depressed and Social Problems scales. Analyses of the SFI showed significantly lower family functioning among the girls with the child abuse history for all scales except for the Directive Leadership. The abused girls also showed significantly lower scores on the Insight scale, and marginally lower Initiative scores at the ARAS. **Conclusions.** These findings may have practical application in the creation of specific preventive and treatment strategies, particularly focused on delinquent tendencies, as well as on enhancing resilience through providing positive environments within families, schools and communities.

Key words:
psychopathology; resilience, psychological; child abuse; adolescent psyhiatry.

Apstrakt

Uvod/Cilj. Zlostavljanje u detinjstvu može biti uzrok različitih psiholoških problema kod odraslih osoba. Malo se, međutim, zna o specifičnim kliničkim i porodičnim profilima, kao i karakteristikama rezilijentnosti adolescenata koji su doživeli zlostavljanje u detinjstvu. Cilj našeg rada bio je ispitivanje simptoma, porodičnog funkcionisanja i rezilijentnosti adolescenata sa iskustvom zlostavljanja u detinjstvu upućenih na psihijatrijski pregled. **Metode.** Uzorak se sastojao od 84 konsekutivno regrutovana mlada ispitanika (prosečne starosti 14,90 ± 3,10, u rasponu od 11 do 18 godina) upućena na prvi pregled u Kliniku za decu i omladinu Instituta za mentalno zdravlje u Beogradu, koji su na osnovu Matrice rizika za zlostavljanje i zanemarivanje dece bili podeljeni u dve grupe. Prvu grupu činili su adolescenti sa iskustvom zlostavljanja u detinjstvu (n = 38, 13 dečaka, 25 devojčica), a drugu, kontrolnu grupu, adolescenti bez iskustva zlostavljanja u detinjstvu (n = 47, 20 dečaka, 27 devojčica). U istraživanju su korišćeni sledeći upitnici: Upitnik za samoprocenu adolescenata (*Youth Self-Report* – YSR), Skala adolescentnih rezilijentnih stavova (*Adolescent Resilience Attitudes Scale* – ARAS) i Upitnik za porodicu, (*Self-Report Family Inventory* – SFI). **Rezultati.** Značajne razlike pronađene su kod adolescentkinja. Na upitniku YSR, zlostavljane adolescentkinje imale su značajno više skorove delinkventnog po-

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našanja i marginalno veće skorove anksioznosti/depresivnosti i socijalnih problema. Analize upitnika SFI pokazale su značajno lošije funkcionisanje kod zlostavljanih adolescentkinja u svim podskalama osim na podskali direktivnog vođstva. Na upitniku ARAS, zlostavljane adolescentkinje imale su značajno niže skorove na podskali uvida i marginalno niže skorove na podskali za inicijativu. **Zaključak.** Navedeni rezultati mogli bi imati praktičnu primenu pri planiranju specifičnih preventivnih strategija i tretmana koji se posebno fokusiraju na delinkventne tendencije kao i na jačanje rezilijentnosti obezbeđivanjem pozitivnog okruženja u okviru porodice, škole i zajednice.

Ključne reči:
psihopatologija; rezilijentnost, psihološka; zlostavljanje dece; psihijatrija, adolescentna.

Introduction

Adolescent victims of any form of previous childhood abuse are at greater risk for developing mental health problems in comparison to young persons who have not been abused [1–6]. As a concept, resilience was introduced in the field of child abuse and neglect in order to encourage investigators to think in terms of protective, rather than risk factors. It has been suggested that a resilient person has the capacity to withstand, overcome or recover from a serious threat [7]. Resilience is also conceptualized as the strength of prosocial skills and emotional regulation [8]. According to Biscoe and Harris [9], being more resilient means having better insight or understanding of the events, independence from others, capacity for forming relationships, initiative to solve problems, more frequent use of humor, creativity and a finer sense of morality. Factors recognized as protective belong to personal, familial and social domains, and the effects of these factors depend on risk constellations and environmental conditions [10], implying the possibility for resilience to be a plastic phenomenon through developmental age, modeled through the interaction of an individual with various environmental experiences [11–13]. Therefore, it may be hypothesized that resilience could have a bidirectional relationship with adverse childhood, and that resilience disturbances in abused adolescents may be different from the decreased resilience in adolescents with non-abuse related psychopathology.

Resilience depends on the supportive family system [14], helping adolescents to successfully adapt to adversity. Overcoming adversities and being resilient are different depending on the presence or absence of consistent, loving, caring mentoring adults who are helping the adolescent to overcome this troublesome period of life. Stable warm relationship with an adult person was found to be a protective factor in development of dissociative pathology and other deleterious effects of childhood abuse such as transgenerational abuse cycle [15].

Previous studies that have investigated the effects of child abuse have shown that victims are in more risk for later psychopathology, including conduct disorder, antisocial personality disorder, aggression, poor self-esteem, cognitive problems, poor academic achievements, anxiety and depression, and suicidal behaviors, compared to non-abused individuals from general population [1, 5, 6, 16]. Other studies compared abused persons with psychological disturbances with more resilient, abused adolescents that did not develop psychiatric symptoms [17]. However, there are insufficient data about specific abuse-related clinical features in adolescent population of first-time psychiatric patients. Furthermore, there are not enough data on resilience and family factors among abused clinical adolescents compared to adolescent psychiatric patients with no abuse history. Such findings would be helpful in differentiating specific effects of child abuse from a wide range of general adolescent non-psychotic psychopathology unrelated to abuse, and give directions for planning specific preventive and therapeutic strategies. Therefore, our study was aimed at investigating clinical symptoms, resilience factors and family functioning in adolescents with the experience of abuse, at their first referral to psychiatric services.

Methods

The study sample consisted of 84 adolescents (33 males, 52 females, mean age 14.90 ± 3.10, ranging from 11 to 18 years) recruited as consecutive first referrals to the Clinic for Children and Youth of the Institute of Mental Health, Belgrade, Serbia, in the period 2006–2010. The first group of participants included adolescents with the experience of abuse in childhood (n = 38, 13 males, 25 females), whereas the second, control, group consisted of non-abused adolescents (n = 47, 20 males, 27 females).

The first, the group of abused adolescents was selected from the Unit for Mental Healthcare of Abused and Neglected Children and Adolescents. Abuse was confirmed according to the Child Abuse Matrices of Risks used in the National Child Abuse Protection Protocol [18]. Most referrals came from regional centers for social work, pediatric units, from the non-abusive family member or adolescents themselves. In the group of abused boys, 5 of them had been exposed to physical abuse, 3 to emotional abuse and 5 boys to both physical and emotional abuse. Six girls, from the total of 25, had been sexually abused, 10 emotionally abused, 3 physically abused and 6 girls had suffered both physical and emotional abuse. In almost all cases of physical and emotional abuse the perpetrators were the victim's father (most frequently in the cases of physical abuse) or mother (most frequently in the cases of emotional abuse). In fewer cases (almost exclusively cases of sexual abuse) perpetrators were the victim's brother or sister (in one case of physical abuse and in two cases of sexual abuse) or the victim's grandfather (one case of sexual abuse), cousin (one case of sexual abuse) or peers (two cases of sexual abuse). The second, non-abused group of adolescents included consecutive first referrals at the Outpatient Department for Children and Adolescents. About 42% of adolescents in the outpatient group were diag-

nosed as having mixed emotional and conduct disorder, 13% with conduct disorder, 35% with depression, and 10% with adjustment disorders. Exluded from the study were adolescents with schizophrenia, schizoaffective and affective psychosis, mental retardation and pervasive developmental disorders. Adolescents from the second group had no experience of abuse according to the Child Abuse Matrices of Risks used in the National Child Abuse Protection Protocol [18].

All study assessment was conducted during psychiatrists evaluation through clinical interviews with adolescents, as well as with the parents.

The two groups were not different in gender or age ($p > 0.05$).

The study was approved by the Ethical Committee of the Institute of Mental Health.

The participating adolescents were further assessed by self-report instruments that were previously adjusted for Serbian population by bidirectional translations and semantic, technical and conceptual analysis: 1) Youth Self–Report (YSR) is a measure of various behavioral and emotional problems in adolescents aged 11–18 years [19]. The questionnaire consists of 112 items and results in 8 syndrome scales (withdrawal, somatic complaints, anxiety/depression, social problems, thought problems, attention problems, delinquent behavior, aggressive behavior, and self-destructive/identity problems), as well as the overall externalizing and internalizing score. Items and scores are gender specific (for example, there is a self-destructive/identity problems scale in YSR only for boys); 2) Adolescent Resilience Attitudes Scale (ARAS) is a self report instrument intended to measure the resilience of adolescents [9]. This 67 items questionnaire includes seven resilience factors: Independence, Insight, Relationships, Initiative, Creativity, Humor and Morality as well as General Resilience defined as persistence in overcoming troubles and belief that troubles can be resolved; 3) Self-Report Family Inventory (SFI) is a 36 items questionnaire intended to assess family functioning [20]. It includes the following factors: Family Health, Conflict, Cohesion, Directive Leadership, Expressiveness. Lower scores represent greater competence on all SFI scales.

Data were separately statistically examined for genders, according to the considerable gender differences in YSR scale definitions. Descriptive data were presented through means and standard deviations for both study groups. Differences were analyzed by the means of multivariate analysis of variance for all the scales of the explored variables, and further on by univariate analysis of variance, if the differences were significant.

Results

The means and standard deviations of the YSR subscale scores for the abused and non-abused clinical groups of boy- and girls are presented in Table 1.

The findings of separate multivariate analyses of variances for boys and girls showed statistically significant differences between the abused and non-abused girls (but not between the abused and non-abused boys) in the clinical population with respect to their mean scores on the Youth Self-Report scales (for girls: Wilks' $\Lambda = 0.69$, $F (8; 42) = 2.42$, $p = 0.03$, multivariate $\eta 2 = 0.32$; for boys: Wilks' $\Lambda = 0.84$, $F < 1$). Therefore, the univariate analyses of variances for each scale of YSR were conducted as follow-up tests only for girls. These analyses showed a significant difference between the means of the abused and non-abused girls on the Delinquent Behavior scale ($F (1; 49) = 4.46$, $MSe = 16.01$, $p = 0.04$, $\eta 2 = 0.08$), with higher values for the abused group, whereas the differences on the Anxious/Depressed and Social Problems scales were marginally significant (Anxious/Depressed scale: $F (1; 49) = 3.82$, $MSe = 59.23$, $p = 0.06$, $\eta 2 = 0.07$; Social Problems scale: $F(1;49) = 3.23$, $MSe = 6.84$, $p = 0.07$, $\eta 2 = 0.06$), with higher scores for the abused females (differences marked in Table 1).

Table 1

Means (and standard deviations) of the Youth Self-Report (YSR) syndrome subscale scores for the abused and non-abused boys and girls, with the difference significance presented for girls

Scale	Boys		Girls		
	abused (n = 13)	non-abused clinical (n = 20)	abused (n = 24)	non-abused clinical (n = 27)	UAV sig.:
Withdrawn	4.00 (2.65)	3.80 (2.50)	5.08 (3.51)	5.00 (2.60)	/
Somatic complains	4.15 (4.00)	3.20 (2.98)	4.71 (3.20)	4.81 (3.01)	/
Anxious/ Depressed	10.15 (8.01)	8.70 (6.04)	14.63 (9.06)	10.41 (6.25)	‡
Social problems	4.15 (2.27)	3.58 (2.49)	4.88 (2.61)	3.56 (2.62)	‡
Thought problems	2.15 (2.44)	2.00 (2.08)	3.79 (3.41)	2.93 (3.16)	/
Attention problems	6.92 (5.04)	7.00 (3.08)	8.71 (4.54)	7.30 (3.78)	/
Delinquent behavior	4.31 (4.52)	5.00 (4.26)	6.00 (4.40)	3.63 (3.61)	*
Aggressive behavior	10.00 (6.90)	9.20 (6.91)	11.29 (6.91)	9.89 (5.98)	/
Self-destructive/Identity problems†	4.62 (4.54)	4.00 (4.53)			/
Internalizing	17.62 (12.61)	15.20 (9.55)	23.25 (13.60)	19.37 (8.69)	/
Externalizing	14.31 (10.93)	14.20 (10.28)	17.29 (10.77)	15.29 (10.00)	/

UAV sig. – Difference significance after univariate analysis of variance (conducted only for girls because the preceding multivariate analysis of variance was non-significant in boys); * – Statistically significant ($p \leq 0.05$); ‡ – Marginally significant ($p \leq 0.07$); / – Not significant ($p \geq 0.07$). †Note: There is a self-destructive/identity problems scale in YSR only for boys.

The means and standard deviations of the ARAS subscale scores for the abused and non-abused clinical groups of boys and girls are presented in Table 2. The findings of multivariate analyses of variances with the subscales of Adoles-

cent Resilience Attitudes Scale as dependent variables, conducted separately for boys and girls, showed significant differences on these subscales between the abused and non-abused adolescents but only in the group of girls (for girls: Wilks' $\Lambda = 0.73$, $F(7;43) = 2.32$, $p = 0.04$, multivariate $\eta 2 = 0.27$; for boys: Wilks' $\Lambda = 0.81$, $F < 1$).

Follow-up tests (univariate analyses of variances for each subscale) were conducted only for the girls. The findings of univariate analyses of variances showed a significant mean difference between the abused and non-abused girls on the Insight subscale of the ARAS, with lower scores among the abused girls, and a respective difference on the Initiative subscale (lower in abused girls) approaching the significance (Insight subscale: $F(1; 49) = 7.92$, $MSe = 138.16$, $p = 0.007$, $\eta 2 = 0.14$; Initiative subscale: $F(1;49) = 3.45$, $MSe = 78.35$, $p = 0.07$, $\eta 2 = 0.07$) (differences marked in Table 2).

Table 2

Means (and standard deviations) of the Adolescent Resilience Attitudes Scale (ARAS) subscale scores for the abused and non-abused boys and girls, with the difference significance presented for girls

Scale	Boys		Girls		
	abused (n = 12)	non-abused clinical (n = 20)	abused (n = 26)	non-abused clinical (n = 26)	UAV sig.:
Insight	61.19 (13.48)	64.00 (13.02)	59.09 (10.96)	68.35 (12.47)	*
Independence	66.48 (12.44)	70.78 (12.43)	57.42 (12.00)	62.39 (9.02)	/
Relationships	69.50 (4.98)	66.50 (10.15)	67.20 (8.93)	67.85 (11.22)	/
Initiative	64.00 (10.23)	67.90 (13.74)	62.32 (6.87)	66.92 (10.40)	‡
Creativity and humor	60.00 (7.72)	60.70 (10.12)	59.68 (9.79)	60.54 (10.50)	/
Morality	67.78 (11.20)	67.08 (8.29)	67.67 (7.58)	68.27 (9.75)	/
General resilience	72.22 (9.92)	73.33 (14.13)	66.49 (13.74)	70.51 (13.84)	/

UAV sig. – Difference significance after univariate analysis of variance (conducted only for girls because the preceding multivariate analysis of variance was non-significant in boys) ; * – Statistically significant ($p \leq 0.05$); ‡ - Marginally significant ($p \leq 0.07$); / – Not significant ($p > 0.07$).

Table 3

Means (and standard deviations) of the Self-Report Family Inventory (SFI) subscale scores for the abused and non-abused boys and girls, with the difference significance presented for girls

Scale	Boys		Girls		
	abused (n = 12)	non-abused clinical (n = 18)	abused (n = 23)	non-abused clinical (n = 27)	UAV:
Family health	55.25 (14.67)	45.67 (13.73)	63.26 (15.87)	49.78 (15.75)	*
Conflict	33.75 (6.20)	27.11 (8.82)	36.43 (10.97)	29.07 (8.48)	*
Cohesion	14.83 (4.43)	12.83 (4.74)	16.91 (4.75)	13.96 (4.39)	*
Directive leadership	8.25 (2.99)	8.06 (2.46)	7.74 (3.02)	8.93 (2.79)	/
Expressiveness	15.33 (4.46)	11.11 (5.54)	17.83 (6.65)	12.00 (5.30)	*

UAV sig. – Difference significance after univariate analysis of variance (conducted only for girls because the preceding multivariate analysis of variance was non-significant in boys); * – Statistically significant ($p \leq 0.05$); / – Not significant ($p \geq 0.07$).

The means and standard deviations of the SFI subscale scores for the clinical groups of the abused and non-abused girls are shown in Table 3.

As in previously presented analyses the mean differences between the abused and non-abused boys with respect to the results on the Self-Report Family Inventory did not reach significance (Wilks' $\Lambda = 0.82$, $F(5; 24) = 1.06$, $p = 0.40$). However, there were significant differences between the abused and non-abused females with respect to their mean scores on the Self-Report Family Inventory (Wilks' $\Lambda = 0.76$, $F(5; 44) = 2.74$, $p = 0.03$, multivariate $\eta 2 = 0.24$). Follow-up univariate analyses of variances showed that abused and non-abused girls were significantly different with respect to the mean scores on all of the subscales of SFI except for the Directive Leadership (Family Health: $F(1; 48) = 9.04$, $MSe = 249.86$, $p = 0.004$, $\eta 2 = 0.16$; Conflict: $F(1; 48) = 7.15$, $MSe = 94.12$, $p = 0.01$, $\eta 2 = 0.13$; Cohesion : $F(1;48) = 5.20$, $MSe = 20.77$, $p = 0.03$, $\eta 2 = 0.10$; Expressiveness: $F(1;48) = 11.88$, $MSe = 35.49$, $p = 0.001$, $\eta 2 = 0.20$), with higher scores in abused girls (differences marked in Table 3).

Discussion

Child and adolescent abuse is a major risk factor for a variety of behavior problems and psychiatric disorders in youth [1] as well as for detrimental physical and psychological problems in adulthood [21]. It is shown that abused persons have a variety of psychopathological symptoms compared to non-abused persons from the general population [16]. In our study, the abused adolescents had clinical specificities in comparison to non-abused ones only among females, reporting significantly more frequent delinquent behavior, and marginally more symptoms of anxiety, depression and social problems. These specificities are in accordance with other findings associating child abuse with depressive symptoms, anxiety, and antisocial behavior [16]. This can be explained by the fact that adolescents with a history of early abuse interact with their friends in a less intimate fashion compared to non-abused adolescents [1, 22], and are more likely to exhibit delinquent behavior [23]. One of the proposed mechanisms is identification with the aggressor [24] that could explain why victims are more prone to aggressive behavior [25] and at higher risk for intergenerational transmission of abuse [26]. The girls

growing up in abusive families develop a kind of "self-preservational" behavior as an act of escape from abusive home-life (delinquency and truancy). They engage in violence in response to their own victimization whereas boys engage in aggressive acts because of other reasons (such as peer pressure) [27]. Our findings might contribute to understanding of the general relationship between abuse and antisocial features, emphasizing it as a potentially pathognomonic dimension of abuse-related psychopathology, not only in comparison to the general population, but also in comparison to non-abused adolescents with psychological disturbances.

The clinical differences found in females in our study may be closely related to the abuse and with the general family dysfunction found among girls. The ones with the experience of abuse had predictably lower family functioning in terms of being less competent (healthy) with more severe conflicts, lower cohesion and with less emotional expressiveness. These findings support previous findings that abused children experience their families as more conflicted and less cohesive. Poor social support may lead to juvenile delinquency [28] and adolescents with high resilient capabilities have more cohesive families, they rely more on immediate family support and have more positive concepts of themselves and their families [29]. Some authors agree that poor parenting skills, parental stress, poor interaction between parents and adolescents, poverty, young parents, parental criminal behaviour or mental health problems and low parental education are connected with more psychological disturbances in their children or adolescents [30]. Similar factors such as young motherhood, lack of positive involvement, low empathy, unstable home environment have been related to abused adolescents [31]. On the other hand, family factors such as stable environment and supportive relationships among family members appear to be linked with resilience [29].

Despite the severe risks, factors of resilience help adolescents thrive and have the ability to successfully adapt to adversity [32, 33]. Regarding the fact that synapses are constantly remodeled following significant experience in a permanently renewed manner [34], resilience factors may be closely, bidirectionally related to the child abuse. Therefore, we hypothesized that resilience disturbances in abused adolescents may be different from the disturbed resilience in adolescents with psychopathology unrelated to abuse. Our findings support this assumption, showing that abused girls had significantly lower insight – the ability to sense, know and understand, and marginally lower initiative, the capacity for problem solving with goal directed behavior. This is in accordance with the assumption that insight and initiative may relate closely to the phenomenon of personal control, previously hypothesized as the key factor of well-being and resilience following childhood abuse [21]. There may be a reciprocity of the level of insight to the tendency to dissociation that is found to be an important consequence of child abuse [35], with the role of protecting the ego-function by decreasing experience of active involvement in the adverse situation. Thus, lower insight may be patognomonic of abuse-related vulnerability in comparison with the vulnerability of non-abused adolescents.

Among male adolescents, we found no differences in any of the examined variables. This may be related to the smaller number of the abused boys which may underestimate the significance of differences that, on some scales, were found to be similar to the female subsample but without statistical confirmation. Another explanation could be related to differences in male and female vulnerability to psychopathology. For various biological and social reasons, males are more prone to disturbances before birth, to accidents or violence victimization, and have a shorter average lifespan than females [36]. They are also more likely than females to have pervasive developmental disorders [37]. This specific gender vulnerability may result in males having stronger adverse reaction to different kinds of negative stimuli that produce psychopathology, related or not related to child abuse.

There are some limitations of this study. All types of abuse were aggregated in analyses, because of the small frequencies of various abuse forms. Furthermore, analyses in a smaller sample of males may have resulted in significance underestimation. Even though age of study participants may be of particular relevance when it comes to resilience and effects of abuse and neglect, due to the very small sizes of specific age groups in this study it was not possible to differentiate the effects of abuse and/or neglect between them and determine if any statistically significant differences exist. In addition, part of the assessment was based on psychiatrists' evaluation through clinical interviews with adolescents, as well as with the parents who, in cases of child abuse could have reported less reliable information and cooperation, emphasizing the need for multi-informant reports about the adolescent behavior in different settings.

Future research should include multi-informant studies with larger sample for both genders and for different types of child abuse, which could give the possibility to examine these factors as covariates in multivariate analyses. Furthermore, future studies could bypass the age limitation by using larger samples or by focusing on specific age groups. Also, analyses could engage additional factors, such as interests and enjoyment in school, including the out-of-family relations with peers and other important persons, as well as non-abusive traumatic events.

Conclusion

Our results show a specific clinical, family and resilience profile for abused adolescent females at their first referral to psychiatric service, compared to their non-abused, first referred peers of the same gender, whereas such specificity was not found among males. These findings may have practical implications in terms of greater focusing on delinquent tendencies among young victims of child abuse (especially females), while the resilience could be enhanced by encouraging the creation of positive environments within families, schools and communities.

The assessment of risk, protection and resilience may help in planning early intervention strategies aimed at pre-

venting abuse and neglect and its adverse outcomes such as behavioral and emotional problems. Early intervention programs that successfully target a number of specific risk and protective factors may contribute to prevention of multiple problems, increasing the chance for better outcomes for every adolescent victim of abuse and neglect.

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Effect of neuropeptide Y on norepinephrine-induced constriction in the rabbit facial artery after carotid artery occlusion

Efekat neuropeptida Y na konstrikciju facijalne arterije kunića izazvane norepinefrinom posle okluzije karotidne arterije

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Abstract

Background/Aim. Atherosclerotic-occlusive changes could be observed in orofacial branches of the external carotid artery. Atherosclerosis-induced ischemia caused alteration in production and release of endothelial factors. The aim of this study was to investigate the influence of carotid artery occlusion (10, 30 and 60 min) on vascular effects of norepinephrine (NOR) and neuropeptide Y (NPY) in the isolated glandular branch of the rabbit facial artery, the main feeding artery for the submandibular gland. **Method.** Changes in isometric tension were recorded in organ bath studies with arterial rings, before and after carotid artery occlusion. **Results.** Concentration-dependent vasocontractile effect of NOR was significantly augmented after 30 and 60 min of carotid occlusion, but only in the rings with intact endothelium. Given alone, NPY showed no effect in isolated glandular branch of the rabbit facial artery, but enhanced NOR vasoconstriction in all the investigated rings. NOR vasocontractile effect enhancement in the presence of NPY was attenuated after 30 and 60 min of carotid occlusion. Also, enhancement of NOR vasoconstriction by NPY was significantly higher in endothelium-intact rings compared to endothelium-denuded rings obtained after 30 and 60 min of carotid occlusion. **Conclusion.** The present investigation provides results of increased vasocontractile effect of NOR and decreased enhancing effect of NPY on NOR vasoconstriction in the rabbit facial artery after carotid occlusion that is related to altered endothelium function.

Key words:
neuropeptides; norepinephrine; carotid arteries; carotid stenosis; rabbits; vasoconstriction.

Apstrakt

Uvod/Cilj. Pokazano je da se okluzivne promene aterosklerotične prirode mogu opaziti na orofacijalnim granama spoljne karotidne arterije. Ishemija izazvana aterosklerozom, dovodi do poremećaja stvaranja i oslobađanja faktora poreklom iz endotela. Cilj ovog istraživanja bio je da se ispita uticaj okluzije karotidne arterije (10, 30 i 60 min) na vazokontraktilne efekte norepinefrina (NOR) i neuropeptida Y (NPY) na izolovanoj žlezdanoj grani facijalne arterije kunića, glavne dovodne arterije za submandibularnu žlezdu. **Metode.** U kupatilu za izolovane krvne sudove ispitivane su izometrijske promene tonusa arterijskih preparata, pre i posle okluzije karotidne arterije. **Rezultati.** Koncentracijski-zavisan vazokontraktilni efekat NOR bio je značajno veći posle 30 i 60 min karotidne okluzije, ali samo na preparatima sa očuvanim endotelom. Primenjen u rastućim koncentracijama, NPY nije imao efekta na tonus izolovane žlezdane grane facijalne aterije kunića, ali je povećao vazokontraktilni efekat NOR na svim ispitivanim preparatima. Povećanje vazokontraktilnog efekta NOR u prisustvu NPY bilo je značajno manje posle 30 i 60 min okluzije. Takođe, efekat NPY posle karotidne okluzije bio je značajno veći na preparatima sa očuvanim endotelom u odnosu na preparate sa uklonjenim endotelom. **Zaključak.** Ovo istraživanje pokazalo je povećanje vazokontraktilnog efekta NOR i smanjenje potencirajućeg efekta NPY na vazokonstrikciju izazvanu NOR na facijalnoj arteriji kunića posle okluzije karotidne arterije koje su povezane sa promenjenom funkcijom endotela.

Ključne reči:
neuropeptidi; noradrenalin; aa. carotis; okluzija; zečevi; vazokonstrikcija.

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Introduction

Salivary gland blood flow, mainly controlled by the parasympathetic and sympathetic nervous system, significantly contributes to salivary secretion. Cholinergic neurotransmitter, acetylcholine (ACh) and adrenergic, norepinephrine (NOR), are mainly responsible for vasodilatory and vasoconstrictory responses in salivary glands, but also for the important role in vascular regulation has non-adrenergic noncholinergic system involving: vasodilators such as vasoactive intestinal polypeptide (VIP) and nitric oxide (NO), and vasoconstrictors such as neuropeptide Y (NPY) and adenosine triphosphate (ATP) [1–3]. Immunohistochemistry revealed NPY to be present around blood vessels and secretory parts in rat and human submandibular gland, colocalized with NOR in sympathetic nerves [4, 5]. Previous studies showed that NPY acts as vasoconstrictor and enhances the response to various constrictor substances in a number of animal and human arterial beds [6–8] . Moreover, some studies emphasize the significance of NPY as a mediator strongly activated under conditions of oxygen deprivation such as in ischemic coronary artery disease [9, 10] . It is important to note that in nonischemic tissues, vascular effect of NPY is mainly a result of activation of NPY Y1 receptors, but under ischemia there was an induction and upregulation of NPY Y2 receptors, mainly involved in non-vasocontractile and angiogenic activities of NPY [3, 11] .

Cellular function depends upon adequate oxygen supply. Oxygen deprivation as the result of ischemia of the human vascular tissue plays the critical role in development and progression of ischemic disorders [12]. It is interesting to note that atherosclerotic-occlusive changes could be observed in human orofacial arteries which maintain local blood flow in salivary glands , such as facial, maxillary and lingual arteries, branches of external carotid artery. Under experimental conditions, Vág et al. [14] have shown that carotid artery occlusion is followed by a decrease of submandibular blood flow in rat, and associated with decreased NO synthesis/release in intraglandular blood vessels. Ischemia affects vascular function in terms of impairment of endothelial function and alteration in production and release of endothelium-derived vasodilators and vasoconstrictors, in favor of the last one [15–18]. A previous study in isolated glandular branch of rabbit facial artery (feeding artery for submandibular gland), after acute carotid occlusion, revealed a decreased responsiveness to both, ACh-endothelium-dependent and VIP-endothelium-independent vasorelaxation after carotid occlusion as a result of impairment of transduction signals including NO, prostaglandins and cAMP [19].

Having in mind the impact of ischemia on endothelial cell function and impairment of vasodilatory responses to ACh and VIP in rabbit facial artery, we hypothesized that acute ischemia would change sympathetic control of vascular tone causing potentiation of vasocontractile responses of NOR and NPY in isolated glandular branch of rabbit facial artery. To test this hypothesis, we investigated the impact of 10, 30 and 60 min of carotid occlusion on vasocontractile effects of NOR and NPY, as well as involvement of endothelium in these effects.

Methods

Animals and organ bath studies

The study was approved by the Ethical Committee of the Faculty of Dental Medicine at the University of Belgrade. The experiments were conducted on Chincilla rabbits (17 males and 5 females), aged 3 months, weighing 2.5–3.0 kg. The animals were anesthetized using urethane (1g/kg *iv*). Experimental ischemia was induced by left or right common carotid artery occlusion by cords for 10, 30 or 60 min. After the mentioned periods, the segments of occluded glandular branch of facial artery and contralateral, nonoccluded (controls) were dissected out and placed in Krebs-Ringer bicarbonate solution (37°C, pH = 7.4 gassed with 95% O_2 and 5% CO_2). The endothelium was removed in some rings by wire. After 60 min of equilibration, 1.0 g tension was applied and segments stabilized for further 30 min. A Hugo Sachs model MC 6621 recorder was used for isometric tension changes registration.

Experimental protocol

Endothelium removal and functional integrity of arterial segments were confirmed by the inability of segments to relax (< 70%) to ACh (10 μM) and by the ability to contract to potassium-rich Krebs-Ringer solution (KCl = 60 mM). Concentration-response curves to NOR (0.1–10 μM), alone, and 30 min after incubation with NPY (0.1 μM), were obtained in endothelium-intact and endothelium-denuded arterial segments. In another group of experiments, vascular effect of cumulative concentrations of NPY (0.01–0.3 μM) was investigated.

Drugs

All the compounds were obtained from Sigma–Aldrich, St. Louis, USA. All the drugs were dissolved in distilled water and prepared as the final concentrations for the 150 μL of bath solution.

Statistics

The vasoconstriction induced by each concentration of NOR and NOR+NPY was expressed as a percent constriction of Krebs-Ring solution (KCl = 60mM)-induced maximal constriction. The maximal effect (E_{max}) and the concentration of the agonist which produced half of E_{max} (pEC_{50} = - log EC_{50}) for each concentration-response curve were obtained in nonlinear regression analysis (GraphPad Prism software). The results were expressed as $\bar{x} \pm$ S.E.M.; n refers to the number of experiments. The results of comparison of vascular effects of NPY, in the control and the occluded rings, were calculated as differences of area under the concentration–response curves (AUC) for NOR, obtained in the presence or absence of NPY, in control and experimental situation. In this way, we were able to integrate effects of NPY and carotid occlusion, both affecting vasoconstriction. The AUC was calculated from each cumulative concentra-

tion-response curve before, and after 10, 30 and 60 min of carotid occlusion (GraphPad Prism software). The results were analyzed using Student's *t*-test for paired and unpaired observations and analyses of variance (one way ANOVA followed by a Dunnet's *post hoc* test or two way ANOVA followed by a Bonferroni's correction). The significance was considered from a value of $p < 0.05$.

Results

NOR (0.1–10 μM) induced concentration-dependent constriction in the rabbit facial arterial rings with and without endothelium is shown in Figure 1. The maximal vasocontractile effect of NOR (10 μM) was significantly augmented in endothelium-denuded compared to endothelium-intact rings but with no change in pEC_{50} (100.5 ± 5.5%; pEC_{50} = 6.07 ± 0.07 compared to 76.0 ± 4.2%; pEC_{50} = 5.91 ± 0.02, respectively). After 30 and 60 min of carotid occlusion, significant augmentation of maximal vasocontractile effect (E_{max}) to NOR was observed in endothelium-intact but not in denuded rings, while pEC_{50} remain unchanged (Tables 1 and 2). The values of AUC for NOR-induced vasoconstriction in endothelium-intact rings were augmented also in rings after 30 and 60 min, but not after 10 min of occlusion (Figure 2).

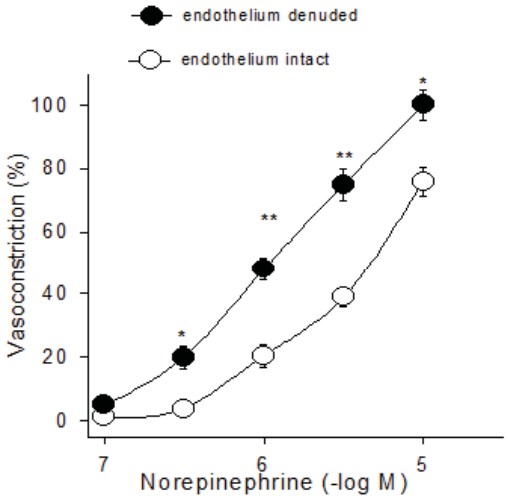

Fig. 1 – Concentration-response curves for norepinephrine (NOR) in the rabbit facial arterial rings with the intact (○) and denuded endothelium (●). Each point represents the x̄ ± S.E.M from 5 experiments. The responses are expressed as percent contraction of maximal contraction induced by 60 mM KCl. *$p < 0.05$; **$p < 0.01$ endothelium-denuded in comparison with endothelium-intact rings (Student's *t* test for unpaired observations).

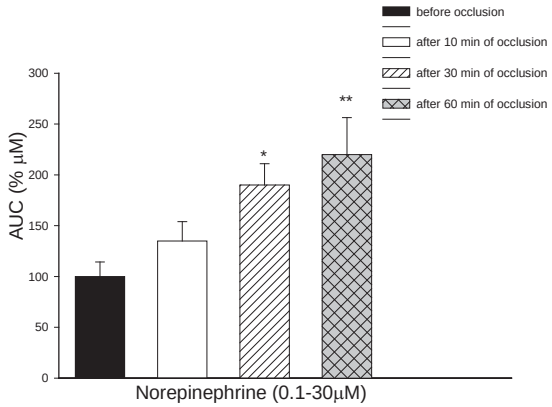

Fig. 2 – The area under curve (AUC) values for norepinepheine (NOR) on endothelium-intact rabbit facial arterial rings before and after 10, 30 and 60 min of carotid occlusion. Each column represents the x̄ ± S.E.M. from 5 experiments. *$p < 0.05$; **$p < 0.01$ compared to the AUC for NOR vasoconstriction obtained in rings before carotid occlusion (one-way ANOVA with Dunnet's *post hoc* test).

Given in increasing concentrations, NPY (0.01–0.3 μM) showed no effect on the resting tone of the isolated glandular branch of the rabbit facial artery, before and after carotid artery occlusion, regardless the endothelium presence.

Table 1

Maximal effect (E_{max}) of norepinephrine in endothelium-intact and -denuded rings before and after 10, 30 and 60 min of carotid artery occlusion

Carotid artery occlusion (min)	E_{max} (%), x̄ ± S.E.M.	
	endothelium-intact rings	endothelium-denuded rings
0	76.0 ± 4.2	100.5 ± 5.5†
10	82.6 ± 4.5	108.7 ± 6.3†
30	131.5± 5.6†*	90.4± 4.5
60	145.5 ± 4.5†**	107.4 ± 6.5

***$p < 0.05$; **$p < 0.01$ (rings before *vs* rings after carotid occlusion); †$p < 0.05$ (endothelium-intact *vs* -denuded rings).**

Table 2

pEC_{50} values of norepinephrine in endothelium-intact and -denuded rings before and after 10, 30 and 60 min of carotid artery occlusion

Carotid artery occlusion (min)	pEC_{50}	
	endothelium-intact rings	endothelium-denuded rings
0	5.91 ± 0.02	6.07 ± 0.07
10	5.90 ± 0.05	6.00 ± 0.03
30	6.00 ± 0.04	5.86 ± 0.07
60	6.09 ± 0.03	5.95 ± 0.03

EC_{50} – concentration producing half of maximal effect (E_{max})

In the presence of NPY (0.1 μM), vasocontractile effect to NOR was significantly enhanced in all the investigated rings obtained before and after 10, 30 and 60 min of carotid occlusion (data not shown). The comparison of dAUC values showed that enhancement of NOR vasocontractile effect in the presence of NPY was attenuated after 30 and 60 min of occlusion, in endothelium-denuded rings, as well as after 60 min of occlusion in endothelium-intact rings. Also, enhancing effect of NPY was significantly higher in endothelium-intact compared to endothelium-denuded rings obtained after 30 and 60 min but not after 10 min of carotid occlusion (Figure 3).

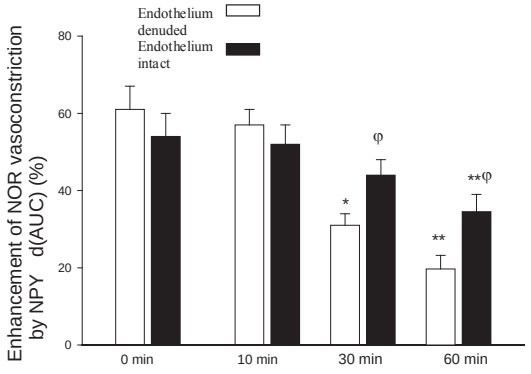

Fig. 3 – Potentiation of norepinepheine (NOR)-mediated vasoconstriction in rabbit facial arterial rings [calculated as the percentual differences of the area curve (AUC) for NOR] in the presence of neuropeptide Y (NPY), before and after 10, 30 and 60 min of carotid artery occlusion. Each column represents the x̄ ± S.E.M. from 6 experiments. *$p < 0.05$, **$p < 0.01$ compared to d (AUC) (%) obtained in the rings before carotid artery occlusion; $^{\varphi}p < 0.05$ endothelium-denuded in comparison with endothelium-intact rings (two-way ANOVA with Bonferroni correction).

Discussion

The results of the present functional study add new insights into the impact of different time of carotid artery occlusion duration as well as of endothelium on vascular effects to NOR and NPY in the glandular branch of the rabbit facial artery. Our study showed that NOR-induced concentration-dependent vasoconstriction in the glandular branch of the isolated rabbit facial artery was significantly enhanced in endothelium-denuded compared to endothelium-intact arterial rings. These results suggest that under non-ischemic conditions in this artery, the presence of endothelium influences the effect of NOR and partly masks it, most probably as a result of endothelium release of vasodilator substances, as seen in other arterial beds [20, 21]. Carotid artery occlusion affects the vasocontractile effect of NOR only in endothelium-intact rings and time-dependently: 10 min was without effect while it was most pronounced after 60 min of carotid occlusion. In the endothelium-intact rings, but not in the denuded, after 30 and 60 min of carotid occlusion vasocontractile effect of NOR was found to be significantly enhanced compared to the matched rings obtained before carotid occlusion. Furthermore, the present results show that carotid occlusion affected responsiveness, measured by E_{max}, but no sensitivity, measured by pEC_{50}, to NOR. It implies that ischemic enhancement of the effect of NOR is rather a result of altered signalling in dysfunctional endothelium than an alteration at the receptor level suggesting the significant role of ischemia-altered endothelium in vasoconstriction induced by NOR in rabbit facial arterial rings after carotid occlusion. Moreover, the fact that in endothelium-denuded rings the effect of carotid artery occlusion on NOR vasoconstriction was not observed, implies that vascular smooth muscle-derived vasoconstrictors are not involved in the observed ischemic alteration of the effect of NOR.

It is well-known that, under physiological conditions, the resting arterial tone is under the control of endothelium and balanced release of vasodilators and vasoconstrictors derived from endothelium, while under conditions of ischemia, this balance is altered in favor of vasoconstrictors due to endothelial dysfunction [22]. Recently, in the glandular branch of the rabbit facial artery, the results showed that 30 and 60 min of carotid occlusion resulted in the impairment of endothelium function and decreased ACh-endothelium-dependent vasorelaxation [19]. The suggested underlying mechanism included a decrease in endothelial NO production/release and a concomitant increased involvement of cyclooxygenase (COX) [19].

Having in mind the increased function of NPY under ischemic conditions [9] we investigated the impact of experimentally-induced ischemia on NPY effect in the glandular branch of the rabbit facial artery. In the present investigation, NPY, given alone in increasing doses, had no effect in the glandular branch of facial artery neither before nor after carotid occlusion. However, NPY significantly increased NOR effect in this artery, under ischemic and non-ischemic conditions, regardless of endothelium presence. Thus, NPY alone is not a potent vasoconstrictor in the rabbit facial artery, but requires a tone to contract, similarly to some other arterial beds such as mesenteric artery of rat and ear artery of rabbit [23, 24]. Comparison of dAUC values showed that under ischemia, enhancing effects of NPY on NOR vasoconstriction decreased in the isolated rabbit facial artery. One possible reason for this could be lying in the fact that under ischemia, expression and activity of NPY Y2 receptors in vascular smooth muscle are increased [11] and that, contrary to activation of NPY Y1, activation of these receptors opposes constriction [25]. Although NPY-mediated enhancement of NOR vasocontractile effect shows no difference in rabbit endothelium-intact compared to denuded facial rings before carotid occlusion, after 30 and 60 min of occlusion, enhancement of NOR vasoconstriction by NPY is higher in endothelium intact compared to denuded rings. This could be due to increased endothelium-release of vasoconstrictors which could contribute to NPY effect , but some studies also point out the fact that endothelium *per se* could synthesize NPY or internalize sympathetically-derived NPY and release it under some circumstances, such as artery occlusion [26, 27].

Conclusion

Having in mind the significance of sympathetically-regulated salivary gland blood flow for salivary gland function, our results provide some new findings concerning NOR and NPY vascular effects on the main feeding artery for submandibular gland under ischemic conditions. This could be important for clarifying mechanisms of endothelial dysfunction underlying salivary gland diseases related to ischemic circulatory disorders.

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ORIGINAL ARTICLE

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Are there any association between polycistic ovary syndrome and congenital abnormalities of Müllerian ducts

Da li postoji udruženost sindroma policističnih ovarijuma i urođenih anomalija Milerovih kanala

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Abstract

Background/Aim. There are many specificities of merital infertility and sometimes surprising connections between some thinks with no connections at first sight. Examinations of these patients imply diagnostic actions such as the blood basal hormone sample, doing hysterosalpingography, ultrahysterosonography, ultrasound examinations, and sometimes laparoscopy and hysteroscopy if there are necessary. The aim of the study was to determine the characteristics of the connection between policystic ovary (PCO) syndrome (Sy) and congenital Müllerian ducts abnormalities. **Methods.** This study included 356 patients treated in the period from January 1, to December 31, 2009, in the Department of Infertility of the Clinic for Obstetrics and Gynecology in Niš, Serbia. Exclusion criteria were no myoma, ovary cysts, tubal and male factors of infertility. **Results.** A total of 180 patients were divided into 3 groups: the group I with PCO sy, the group II with uterine congenital malformation and the group III with a combination of these disorders. The middle age of patients was 29.6 ± 4.8, body mass index (BMI) was 26.1 ± 4,8 kg/m² the middle thicknes of endometrium was 5.2 + 2.7 mm, and there were no significant differences between the examined groups. There were no significant among in a number of miscarriages in the examined groups. We found that PCO Sy and congenital abnormalities of Müllerian ducts were conjoint in 30% of examined patients. **Conclusion.** Conjoined PCO Sy and congenital abnormalities of Müllerian ducts do not result in a higher number of misscarriages than only either PCO Sy or abnormalities of Müllerian ducts. It is important to check BMI, basal level of follicle stimulating hormone and number of antral follicles because the induction protocol and concentracion of inductors depends on these characteristics, thus, the succsessful cycles and pregnancy.

Key words:
polycystic ovary syndrome; uterus; congenital abnormalities; comorbidity; risk assessment.

Apstrakt

Uvod/Cilj. Postoje mnoge specifičnosti u ispitivanju bračnog steriliteta koje ponekad mogu da nas iznenade svojom pojavom, povezanošću ili mogućom zajedničkom genskom ekspresijom. Tako, moguća je i povezanost sindroma policističnih ovarijuma (PCOS) i kongenitalnih malformacija Milerovih kanala. Cilj rada bio je da se utvrde karakteristike povezanosti PCOS i kongenitalnih malformacija Milerovih kanala. **Metode.** Studijom je bilo obuhvaćeno 356 infertilnih žena lečenih u periodu od 1. januara 2008. do 31. decembra 2009. u Ginekološko-akušerskoj klinici u Nišu. Pacijentkinje sa miomima, cistama jajnika, tubarnim sterilitetom, kao i infertilni muškarci nisu uključeni u studiju. **Rezultati.** Ukupno 180 pacijentkinja bilo je podeljeno u tri grupe: grupa sa kongenitalnim malformacijama Milerovog kanala, grupa sa PCOS i grupa sa udružene obe pojave. Prosečna starost pacijentkinja iznosila je 29,6 ± 3,7 g, srednja vrednost indeksa telesne mase (BMI) 26,1 ± 4,8 kg/m2, a srednja vrednost debljine endometrijuma 5,2 ± 2,7 mm. Nije utvrđena statistički značajna razlika među grupama za navedene parametre izuzev za BMI. Nije bilo značajne razlike u broju pobačaja među ispitivanim grupama. Kombinacija PCOS i kongenitalnih malformacija Milerovih kanala utvrđena je kod 30% ispitivanih pacijentkinja. **Zaključak.** Udruženost ispitivanih pojava nema uticaja na povećanje incidencije spontanih pobačaja, ali treba obratiti pažnju na prateće faktore kao što je BMI, bazalni uzorak folikulostimulirajućeg hormona i broj antralnih folikula jer oni, takođe, govore o kvalitetu jajnih ćelija koje sazrevaju.

Ključne reči:
jajnik, policistični, sindrom; materica; anomalije; komorbititet; rizik, procena.

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Introduction

In everyday work sometimes some specific and uncommon thinks at first sight can be found. Examinations such as blood basal hormone samples, hysterosalpingography, ultrahysterosonography, ultrasound examinations and sometimes laparoscopy and hysteroscopy are perfomed sometimes. But there are some investigations with the aim to find the connections between polycystic ovary syndrome (PCO sy) [1] and congenital abnormalities of Müllerian ducts [2] as well as different genes expressions. In that case there is no difference between mild or other forms of congenital Müllerian abnormalities. Genes which are maybe involved are WNT genes probably WNT 4. WNT4 gene has influence on growth factor for development of kidney, adrenal glands, mammary glands, pituitary glands and female reproductive tracts. With late development of that gene locus, masculasations happens in female fetus as well as stronger steroidogenesis [3]. WNT5, WNT7, HOXA 10 and HOXA 11 genes locus have a very important part in regulation uterus stroma as well as production estrogens and progesterons. WNT and HOXA genes are both important in developing anteroposterior axis of reproductive tract. It means that in special conditions their expression influences the different development of Müllerian ducts and ovaries.

The aim of the study was to determine if the connections between PCO sy and congenital Müllerian ducts abnormalities exist.

Methods

This study included 356 patients treated in the period from January 1, 2008 to December 31, 2009, in the Department of Infertility in Clinic for Obstetrics and Gynecology in Niš. All the patients with myoma, ovary cists, tubular and male factors of infertility were excluded. A total of 180 patient left, so they were divided into 3 groups: the group I with PCO sy, the group II with uterine congenital malformations and the group III with a combination of these disorders.

The patients were examined using the protocols: basal blood hormone sample, hysterosalpingography or ultrahisterosonography. If the diagnosis of PCO sy was made oral insulin glucose test with the level of insulin, ultrasound examination, and endoscopic procedures, if necessary, were also performed.

Also, we investigated variables like body mass index (BMI), age, as well as the number of miscarriages.

All the results were statistically analyzed and shown as mean values standard deviation. The difference was statistically significant when $p < 0.05$.

Results and discussion

The percentage of association between PCO sy and Müllerian duct congenital abnormalities was about 30 but as we cannot find the similar datas in the literature, it is hard to compare. The clinical and epidemiological features of the group of patients with PCO sy and the group with the combination of PCO sy and congenital Millerian abnormalities are shown in Table 1. There is no significant difference between the examined groups in any variables except BMI (Table 2). Also, there were no significant differences in a number of misscarrigies among the groups (Table 3). If we compare congenital Müllerian abnormalities in our study (Table 4) and the literature data the percentage of them is similar, except uterus bicornis and arcuatus which differs a little; there were more bicornis than other abnormalities. Usually, the biggest prevalence had uterus arcuatus (Table 5). It could be the result of

Table 1

Clinical and epidemiological characteristics of the patients

Characteristics	PCO sy and congenital Müllerian abnormalities	PCO sy	Total
Patients (n)	60	60	120
Age (years) x ± SD	29.7 ± 3.5	30.1 ± 3.6	29.6 ± 3.7
BMI (kg/m^2), x ± SD	25 ± 0.46	27.1 ± 4.5	26.1 ± 4.6
Duration of infertility (years), x ± SD	3.4 ± 2.4	3.6 ± 2.3	3.5 ± 2.4
Endomerial thickness 2–4 days (mm), x ± SD	4.8 ± 2.2	5.8 ± 3.2	5.2 ± 2.7
FSH 2–4.days (miV/mL), x ± SD	6.1 ± 2.0	5.9 ± 1.9	5.2 ± 2.7
The antral follicle count (n), x ± SD	18.3 ± 14.2	21.5 ± 16.2	20.2 ± 15.7

PCO sy – policystic ovary syndrome; BMI – body mass index; FSH – follicle stimulating hormone.

Table 2

Body mass index (BMI) in the studied groups of patients

Groups of patients	BMI (kg/m^2)		
	< 20	20–29	> 20
PCO Sy, n	6*	49	5
Mixed, n	0	55	5

PCO sy – policystic ovary syndrome; Mixed – PCO sy and congenital Müllerian abnormalities; *PCO sy *vs* mixed $p < 0.05$ (statistically significant).

Table 3

The number of miscarriages in the studied groups of patients

Groups of patients	Miscarriages		
	One	Two	≥ Three
Mixed, n (%)	3 (5)	2 (3.3)	2 (3.3)
PCO Sy, n (%)	6 (10)	1 (1.7)	0 (0)

PCO sy – policystic ovary syndrome; Mixed – Müllerian ducts abnormalities conjoined with policystic ovary syndrome.

Table 4

Congenital anomalies of Müllerian ducts in the different groups

Group of patient	Anomalies					
	Arcuatus	Bicornis	Unicornus	Duplex	Subseptus	Septus
Mixed, n (%)	24 (40)	20 (30.3)	2 (3.3)	2 (3.3)	10 (16.5)	2 (3.3)
Müllerian ducts abnormalities, n (%)	24(40)	24 (40)	4 (6.6)	4 (6.6)	4 (6.6)	4 (6.6)

Mixed – Müllerian ducts abnormalities conjoined with policystic ovary syndrome.

Table 5

The number of miscarriages depending on the type of anomaly

Anomalies	Miscarriages (n)	
	Mixed group	Müllerian abnormalities group
Arcuatus	2	2
Bicornus	2	1
Unicornus	0	0
Duplex	0	2
Subseptus	1	1
Septus	0	1

Mixed – Müllerian ducts abnormalities conjoined with policystic ovary syndrome.

different diagnosis procedures such as hysterosalpingography, hysteroscopy, ultrasound, and it is also possible that the investigator can have his/her personal opinion.

There are no significance differences between miscarriages in the group with PCO sy and the group PCOsy and Müllerian congenital abnormalities, so it means that perinatal outcome is not worse if we have there two problems conjoined. It means that if we make good diagnostic procedures we can treat PCO sy as well as congenital Müllerian abnormalities and have good results. Literature data have reported successful results between 10% and 65%, our were about 11%.

Some studies prove a significant difference between those with uterine malformations and those with normal uterus in the abortion rate, and preterm deliveries. It is expected, but we did not have a group with the normal uterus because it was not the aim of our study. The patients with didelphys and unicornuate uterus have similar effect on reproduction, because we can considered that didelphys is symmetrical duplication of unicornuate uterus. Patients with bicornuate uterus also have a poor pregnancy outcome, so patients with partial bicornis have a better pregnancy outcome than patients with a complete one. Even if there are no differences between the number of miscarriages, the highest number is in the group with PCO sy with one miscarriage, two had only one, and more than two none. It means that patients after one miscarriage go to concluding and other diagnostic procedures (ultrasound or hysteroscopy) as well as resection of septum, metroplasty or medicaments for PCO sy.

It seems that the patients with uterine malformations have high abortion and delivery rates as well as low delivery rates from their first pregnancy to the every next pregnancy.

It is very interesting, if there are any differences between BMI in different groups, what are the values of basal blood hormone samples, and the measurements of endometrium on the day 4 following ultrasound. Our results demonstrated that the middle age of patients was 29.6 ± 3.7, BMI 26.1 ± 4.8 kg/m^2 and the middle thicknes of endometrium 5.2 ± 2.7 mm with no significant differences among the groups.

The number of miscarriages was equal in the groups and similar to the literature data (from 10% to 60%), and if similar multicentric investigatations are performed, we can have good results, for instance higher number of ovulations can improve perinatal outcome. It means that the improved quality of ovarian cells will result in good ovulation and pregnancy [4]. Some studies have examined the pregnancy outcome in patients with untreated uterine malfomations and their pregnancy rate is exactly the same in their first and later pregnancies as the abortion, and the preterm delivery rates [5–7].

It has been very imoprant to identify all endocrinological, clinical and ultrasound disorders which can influnce ovulations.

We do not speak about the level of basal follicle stimulating hormone (FSH), which is also very important for the protocol of induction of ovulations, as well as the types of drugs and their effects.

BMI, and age are also important. Women with higher BMI could have more antral follicles, but lower intermediate and higher, as we can see in many multicentric studies. It should be kept in mind and, if necessery, to use different protocol of ovulation.

Conclusion

Conjoined PCO sy and congenital abnormalities of Müllerian ducts do not result in the higher number of miscarriages. It is important to check BMI, basal level of FSH and number of antral follicles because the induction protocol and concentration of inductors depend on these characteristics, and consequently the successesful cycles and pregnancy.

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GENERAL REVIEW

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Significance, aetiology and prevention of venous thromboembolism in pregnancy and puerperium

Značaj, etiologija i prevencija venskog tromboembolizma u trudnoći i puerperijumu

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Key words:
venous thromboembolism; pregnancy; postpartum period; risk factors; heparin, low-molecular weight.

Ključne reči:
tromboembolija; vene; trudnoća; puerperijum; faktori rizika; heparin, niskomolekulski.

Introduction

Venous thromboembolism (VTE) complicates only 0.05−0.2% of all pregnancies, yet it is a leading cause of maternal morbidity in the western world, while pulmonary embolism (PE) is the cause of about 10% of all maternal deaths during pregnancy and puerperium [1–11].

Deep vein thrombosis (DVT) and PE are distinct but related aspects of the disease process called VTE [6, 10]. This is a dynamic process which can be presented as acute form, with all clinical symptoms and signs, and as a silent, subclinical, chronic form, with the recurrence rate of 17.5% during the first two years, and 30.3% within eight years after the first episode [12]. More than 60% of women develop chronic venous insufficiency due to postthrombotic syndrome, with leg swelling, pain, ulcerations and varices [11, 13, 14]. The most significant consequence of VTE is massive PE, which may end by sudden death in 20% of cases, leaving no time for any medical intervention [10]. Discovering such a state could be a diagnostic problem, but it was proven to be worth the effort, because appropriate diagnosis, prevention and treatment could reduce mortality rate from 20% to 0.7% [15].

The goal of this article was to analyse the risk factors for VTE in pregnancy and puerperium, and medicational options and protocols for thromboprophylaxis.

Characteristics of venous thromboembolism in pregnancy and puerperium

During pregnancy approximately 80% of VTE are DVT and 20% are PE [6, 8, 9, 16]. Two thirds of pregnancy-related DVTs occur before, and one third after the delivery [1, 4, 17]. Considering the fact that postpartal period lasts much shorter than antepartal (6 : 40 weeks respectively), we conclude that the risk of developing DVT is approximatelly 3 times higher in the postpartum period. The same holds for PE 43−60% develops in the first 4−6 weeks after the delivery [8, 9, 13, 18].

Pregnancy itself is an independent risk factor for VTE (4−5 times higher risk than in non-pregnant women of similar age) and in puerperium the risk is even higher (from 15 up to 60 times) [1, 6, 8, 9, 11, 19–21]. Pregnancy is associated with prothrombotic state, which is one of physiologic changes meant to protect pregnant women from peripartal haemorrhage, which is still the main cause of maternal mortality in the developing world [9, 16, 21]. Beginning early in pregnancy, there is an increase of procoagulant factors and a decrease in the level of protein S, and a resistance to activated protein C [8, 9, 11, 13]. Two other elements of Virchow's triad are also present. Venous stasis is a consequence of mechanical obstruction of blood flow by gravid uterus, and of hormonally induced decrease in venous tone, resulting in slower blood flow in lower limbs. Endothelial damage is a result of tearing and distension during delivery [8, 9, 11, 18].

One of the characteristics of pregnancy-related DVT is 80−90% occurence in the left leg (compared to 55% in non-pregnant patients), which is a consequence of the anatomy of the left common iliac vein [7, 9, 13, 17, 22]. More than 70% of gestational DVTs are located in the ileofemoral region (9% in non-pregnant patients), again predominantly on the left side; they are more likely to embolise (in 40−50% of cases), and are associated with low abdominal pain, mild fewer and leukocytosis [23, 24].

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Risk factors for venous thromboembolism

It is believed today that VTE is a convergence of underlying genetic predisposition − thrombophilia, and of acquired precipitating causes [12].

Inherited thrombophilias

Factor V Leiden (FVL) is thrombophilia with high prevalence in general population; in 20−46% of pregnant women with VTE the heterozygot for FVL is found [11, 13]. FVL is a single base pair mutation on a factor V gene that makes it resistant to inactivation by the activated protein C complex. This results in a loss of one of the normal protective antithrombotic mechanisms [25] and increases the risk of VTE 41 times in homozygous and 2−16 times in heterozygous patients [8, 11]. In the non-pregnant state, FVL deficiency equally leads to DVT and PE; in pregnancy DVT dominates, probably because FVL is here associated with a more adherent and stable thrombus, due to increased local thrombin generation, which reduces the probability of embolisation [11].

Mutation on a 20210 nucleotide on a prothrombin gene (G20210A) increases plasma prothrombin concentration, with consequent increased risk of myocardial infarct, cerebral venous thrombosis and VTE [25]. In pregnancy it increases the risk of VTE 3−10 times, and it is found in 20% of gestational VTE [8].

Hyperhomocysteinemia is rare in pregnancy, because of pregnancy-related physiological reduction in homocysteine [13, 26].

Protein S, protein C and antithrombin (AT III) deficiency are less common. The prevalence of AT III deficiency in women with pregnancy-associated VTE is 1−19%, the prevalence of protein C deficency 2–14%, and for protein S deficiency 1–12% [11]. The risk of pregnancy-associated VTE in cases of AT III deficiency type I (quantitative) was found to be 1 : 2.8, and for type II (qualitative) 1 : 42 patients with no previous history of VTE. This leads to more aggressive thromboprophyllaxis in pregnancy for AT III deficient patients [27]. AT III deficiency mainly leads to DVT, often in uncommon regions: kidney, retina, mesenterium, upper limbs, *vena cava*. In cases of protein C and protein S deficiency the risk of VTE is increased 2−4 and 3 fold respectively [8]. One should keep in mind that there are numerous conditions that influence thrombophilia screen: liver disease, as well as pregnancy itself, lower protein C and S levels; severe infection, nephrotic syndrome, massive thrombosis lower AT III level, so laboratory results should be carefully interpreted [28].

4G/5G sequence polymorphism in PAI 1 (plasminogen activator inhibitor 1) gene promoter: PAI 1 is produced by endothelial, smooth muscle, liver cells and platelets and represents a principal inhibitor of fibrinolysis. Its plasma level steadily increases during pregnancy and at term it is 3 fold higher than in the non-pregnancy state; there is also PAI 2, produced by placenta [29, 30]. They both contribute to hypercoagulable state seen in every pregnancy. DVT risk could be augmented by the presence of mutation changes on 4G/5G base-pair region on PAI 1 gene, that modulate PAI 1 synthesis. 4G allele is too small to bind gene transcriptional repressors, so 4G/4G allele homozygosity leeds to 3−5 fold higher level of circulating PAI 1, and was associated with metabolic syndrome and a greater risk for cardiovascular and thrombotic disease [30–35]. Sartori et al. [36] report a greater risk of thrombosis both in sympthomatic thrombophilic patients (OR 2.85, 95% CI 1.26−4.46) and idiopatic DVT patients (OR 3.1, 95% CI 1.26−7.59) with 4G/4G phenotype. In pregnancy, this disorder is frequently associated with FVL mutation, or antiphospholipid syndrome, or protein S deficiency, further predisposing these women to thrombosis as well as implantation failure [30, 32, 35, 37, 38].

It should be mentioned that the inherited thrombophilias are involved in development of other obstetrics complications associated with insufficient fetomaternal circulation and failure of implantation, such as early and late abortion, preeclampsia, placental abruption, fetal intrauterine growth restriction (IUGR) [25, 39–41].

Inherited thrombophilias are proven to be the cause of gestational VTE in 20−50% [25] and represent a danger that should not be underestimated [2, 3, 7, 16]. On the other hand, thromboprophylaxis itself could be risky for both fetus and mother; so one should be rational, meaning that one should estimate the risk and identify patients who really need thromboprophylaxis. Estimated risk of pregnancy-associated VTE in thrombophylic women without prior VTE (odds ratio) is 34.4 for FVL homozygosity, 8.32 for heterozygosity; 26.6 for homozygous prothrombin gene mutation 6.8 for heterozygous; 4.76 for antithrombin, 4.76 for protein C and 2.19 for protein S deficiency [9, 20, 26, 42, 43] (Table 1).

Table 1

Risk of pregnancy-associated venous thromboembolism (VTE) in women with thrombophilia and without previous VTE

Type of thrombophilia	Relative VTE risk OR (95% CI)	Estimated absolute VTE risk (per 1,000 patients)
AT III deficiency	4.7 (1.3–17.0)	4/1000
Protein C deficiency	4.8 (2.2–10.6)	4/1000
Protein S deficiency	3.2 (1.5–6.9)	3/1000
Factor V Leiden		
homozygous	34.4 (9.9–121)	34/1000
heterozygous	8.3 (5.4–12.7)	8/1000
Prothrombin G20210A		
homozygous	26.4 (1.2–559.2)	26/1000
heterozygous	6.8 (2.5–18.8)	6/1000
MTHFR C677T (homozygous)	0.74 (0.22–2.48)	1/1000

OR – odds ratio; CI – confidence interval; AT III – antithrombin III; MTHFR – methylene tetrahydrofolate reductase.

Based on these estimates, in cases of thrombophilia with no prior VTE the American College of Chest Physicians (ACCP) recommends antepartal low-molecular weight heparin (LMWH) prophylaxis only for homozygous women with FVL or prothrombin gene mutations, who have a positive family history for VTE [26, 43, 44].

At the moment there are no proofs in the literature that justify universal screening for thrombophilias in pregnancy. It is recommended that screening should be selective, conducted in patients with a history of VTE, with first and second degree relatives who had VTE, as well as those with complications in previous pregnancies [45].

Acquired risk factors

The most important among acquired risk factors is the previous VTE [3, 4, 18, 21]. Pregnancy itself amplifies the risk of VTE recurrence 3.5 fold [7, 9, 11, 12, 20]. In their prospective study on 125 pregnant women with prepregnancy VTE Brill-Edvards et al. [46] using only postpartal VTE prophylaxis, have shown that the absolute recurrence rate was 2.4%. No recurrence was observed in 44 women who had no evidence of thrombophilia, and who had experienced VTE related to a temporary risk factor. This study challenged the assumption that all women with the history of VTE should receive antepartal thromboprophylaxis. In their retrospective cohort study Roeters Van Lennep et al. [47] tried to evaluate effectiveness of low doses of LMWH prophylaxis in women with intermediate risk for VTE (during six weeks postpartum) and with high risk (during entire pregnancy and six weeks postpartum). They had 5.5% VTE recurrence, all in the high risk group and concluded that a low dose LMWH might not be sufficient in the high risk pregnant patients. Stratta et al. [48] emphasize that it is important to estimate the risk properly and determine the appropriate LMWH dose and dosing regimen (once/twice daily) for each risk level. Thus, although available data from clinical trials are not completely uniform, it can be concluded that even though pregnancy increases the risk of VTE recurrence, antepartal prophylaxis is not routinely recommended. It should be applied in patients with idiopathic first VTE episode, in the presence of an underlying thrombophilia, in women who used oral contraceptives [45]. Puerperium is by all means a period that demands prophylaxis and, in cases of higher risk, even augmentation of the usual doses. Phabinger et al. [49] studied the risk of VTE recurrence in pregnancy without antepartal thromboprophylaxis and found that antepartal risk was 6.2%, and postpartal 6.5% [41].

Antiphospholipid-antibody syndrome (APLA) leads to recurrent artherial and venous thrombosis, as well as other pregnancy complications (spontaneous abortion, preeclampsia, IUGR) [21]. APLA presence affects almost all haemostatic factors, provoking thrombotic diathesis, complement activation, inflammation, disbalance of angiogenic factors and disturbance of normal fetoplacental development. Anticomplemental and vasomodulatory action of heparin make this drug irreplaceable in prevention of APLA complications in pregnancy [50]. ACCP recommends antepartum administration of heparin combined with a low dose aspirin (75−100 mg/day) [26].

After Caesarean section the prevalence of clinically significant VTE is 0.9%. We should consider thromboprophylaxis in women older than 35 years, with body mass over 90 kg, with present infection, varicous veins, gestational hypertension, multiparity, VTE in anamnesis, emergency Caesarean section or hysterectomy after Caesarean section [12].

Assisted reproductive treatment (ART) also represents a risk factor for VTE, especially when associated with ovarian hyperstimulation syndrome (OHSS). It is believed that this is a consequence of 10 fold increased estradiol levels by hormonal stimulation, increased coagulation factors concentration and decreased fibrinolysis. Thrombosis typically occurs between the 7th and 10th week of gestation, located in about 60% in upper limbs, neck and head veins [7, 12]. In cases of pregnancies after ART routine thromboprophylaxis is not recommended, but in cases of OHSS, LMWH prophylaxis is recommended after a resolution of the syndrome [26].

Preeclampsia was found to increase three fold the risk of VTE in the third trimester and postpartum. Preeclampsia is a consequence of maternal immunologic maladaptation to fetal and placental tissue, resulting in generalised endothelial insufficiency, disturbed eikosanoid metabolism, lipid peroxidation, inflammation, activation of complement system and coagulation cascade. There are numerous studies that connect inherited thrombophilias and preeclampsia [51–55]. Heparin and low-dose aspirin are used to treat procoagulant and inflammatory disorders in such situations [51, 55, 56].

Among other risk factors (advanced age, parity, maternal comorbidity etc) maternal obesity should not be forgotten, because it is a global epidemic nowadays [57]. Hypercoagulability, venous stasis and endothelial disfunction in pregnancy are exacerbated by obesity. Body mass index (BMI) over 30 kg/m^2 increases the risk for VTE 1.5−5.3 fold [5, 7], so it is recommended that all women with morbid obesity (BMI over 40 kg/m^2) should receive seven days postpartal LMWH prophylaxis [4, 45, 57]. In cases of increased body mass higher LMWH doses may be needed, but dose estimation according to actual body weight could lead to overdosing. In such cases it would be wiser to use lean body weight for appropriate dosing, with plasma anti-factor Xa level monitoring [57].

We thought it would be interesting to mention a study of Jakobsen et al. [58], who investigated ante- and postpartal factors for development of VTE. To antepartal risk factors already mentioned in the literature (age older than 35 years, multiple pregnancy, blood group A, obesity, smoking), they added pregnancy after assisted reproductive treatment, gestational diabetes, nulliparity, weight gain in pregnancy less than 7 kg. The authors found that besides the age over 35 years, operative delivery, hypertension, blood group A, postpartal risk factors were emergency Caesarean section, haemorrage or infection, preeclampsia, IUGR, assisted reproductive treatment, smoking. Immobilisation, especially in combination with higher BMI, represents an important risk factor ante- and postpartaly [4, 58].

Medication options for thromboprophylaxis in pregnancy and puerperium

The goal of thromboprophylaxis is to provide VTE protection with minimal side effects for the mother and no effects on the fetus. Although today we have a huge choice of anticoagulant and antiplatelet agents, heparin is still the anticoagulant of choice for VTE prophylaxis and treatment in pregnancy [6, 16, 26, 45, 59].

Heparin, neither unfractionated (UFH) nor LMVH, crosses the placenta; it is not secreted in breast milk, it is not teratogenic and there is no evidence of risk of fetal haemorrhage. Currently, LMWHs have replaced UFH as the first choice anticoagulant [26, 27, 45, 59–63]. LMWHs are at least as effective as UFH, but produce more predictable anticoagulant response due to better bioavailability (90−100% after subcutaneous administration), longer half life (4−6 h), dose-independent renal clearance, decreased affinity for heparin-binding proteins, endothelial cells and macrophages [6, 7, 48]. Effective anticoagulation can be achieved by subcutaneous application of LMWH once daily, with no need for routine laboratory monitoring of anti-factor Xa activity in plasma.

Nevertheless, in certain situations LMWH dose adjustment is necessary and that demands anti-Xa level assessment. Pregnancy changes LMWH pharmacokinetics. Together with the increase of cardiac output and plasma volume, glomerular filtration rate progressively increases from the first trimester; at term it is 50−60% higher than in the non-pregnant state, with increased volume of distribution and drug clearance [48]. This could result in subprophylactic anti-Xa level in 26% of patients [6, 7, 48]. Anti-Xa activity is inversely related to body weight, so LMWH doses should be modified in cases of maternal obesity [14, 57]. On the other hand, in patients with renal disease and creatinine clearance below 30 mL/min, standard LMWH doses may lead to accumulation of heparin, higher anti-Xa level and increased risk of bleeding. In such cases LMWH dose should be reduced (based on anti-Xa level) or UFH used instead [18, 26, 64].

According to dose regimen, LMWH doses used for VTE prophylaxis in pregnancy could be prophylactic (for example dalteparin 5000 units or enoxaparine 40 mg, subcutaneously every 24), intermediate (dalteparin 5000 units or enoxaparine 40 mg, subcutaneously every 12) or adjusted (dalteparin 200 units/kg once daily or 100 units/kg/12 h; enoxaparin 1 mg/kg/12 h) [26, 43, 45] (Table 2).

More favorable anti-Xa/IIa ratio of LMWHs comparing to UFH (2−4 : 1), significantly lowers the risk of haemorrhage (1−2%, and mostly related to obstetric causes) [1, 26, 63]. Prolonged use of UFH increases risk of osteoporosis, with 2−3% incidence of osteoporotic fractures and significant reduction in bone density in up to 30% of patients [26, 64]. After LMWH therapy bone mass reduction equals physiological bone mass reduction in pregnancy [1, 13, 22, 26, 65–68]. Heparin induced thrombocytopenia with or without thrombosis (HIT/HITT), carries 1−3% risk with UFH and is 10 fold less frequent after LMWH than after UFH therapy [26, 66]. The risk is indeed very low in obstetric patients receiving LMWH, so routine platelet monitoring is not recommended [7, 68–71]. Cutaneous allergic reactions (delayed type 4 hypersensitivity reaction) occur more frequently in pregnancy (1.8−29%) than in general population, but they are seldom severe [26]. This problem may be resolved by switching to another LMWH preparation or danaparoid or fondaparinux [1, 72]. One further advantage of LMWH over UFH is significantly less procoagulant "rebound effect" after withdrawal of the therapy [73, 74].

At the time of delivery LMWH advantages may became disadvantages: anticoagulant effect can persist more than 12 h after the last dose and protamin sulfate cannot neutralise its action completely in case of need (anti-Xa effect remains), so peripartal haemorrhage can occur [14, 64, 75–78]. LMWH discontinuation is recommended 24 h before induction of labour or planned Caesarean section [7, 26, 78]. High risk patients can be converted to intravenous UFH (aPTT monitoring required), which can than be stopped 4–6 h before the induction of labour [1, 6, 26, 76]. If haemostasis is adequate, thromboprophylaxis could be continued 6−12 h after vaginal and 12−24 h after Caesarean delivery [1, 4, 7, 9, 26, 45, 76, 78].

With the increased use of neuraxial anesthesia in labor, we should be aware of its possible complication – spinal haemathoma with subsequent paraplegia [79]. In order to reduce the risk of such event, epidural catheter can be safely inserted 12 h after prophylactic and 24 h after therapeutic LMWH dose; it can be remowed 12 h after the last dose [4, 7, 16, 77]. Anticoagulation can be restarted 4–24 h after catheter removal [8, 9, 20, 27, 45, 64, 77, 80].

Warfarin crosses the placenta and has proven teratogenity [6, 26, 45]. Coumarin embriopathy is dose-dependent (more than 5 mg/24 h) and occurs in 5% of cases exposed between the 6th and 12th gestational weeks, so in this period warfarin is contraindicated [45]. The first trimester warfarin exposure leads to abortion; later exposure may be associated with fetal haemorrhage, CNS abnormalities, child's mental

Table 2

Thromboprophylactic low-molecular weight heparin (LMWH) dose regimen in pregnancy/puerperium

LMWH	Subcutaneous doses (for women weighting 50–90 kg)		
	prophylactic (low)	intermediate (moderate)	weight adjusted (high dose)
Enoxaparin	40 mg/24 h	40 mg/12 h	1 mg/kg/12 h or 1.5 mg/kg/24 h
Dalteparin	5000 U/24 h	5000 U/12 h	100 U/kg/12 h or 200 U/kg/24 h
Tinazaparin	4500 U/24 h	4500 U/12 h	175 U/kg/24 h

retardation and increased risk of placental abruption and postpartum haemorrhage [9, 13, 26]. The use of warfarin in pregnancy can be justified in case of women with mechanical heart valves, where benefit overweighs the risk [6, 26]. In all the other cases it should be replaced by heparin as soon as patient finds that she is pregnant [26]. Since it is not secreted in breast milk, warfarin is safe to use during breastfeeding, although it requires close monitoring [6, 13, 26, 45].

Dextran is today practically abandoned in obstetrics, because of the risk of anaphyllaxis associated with uterine hypertonus, profound fetal distress and even death [45].

Danaparoid – experience is limited with its use; crossing the placenta and secretion in breast milk were not found, but still its only indication in pregnancy for the time being is complication of heparin therapy [14, 26, 45]. In the newest ACCP recommendations danaparoid is suggested over lepirudin or fondaparinux for treatment of HIT during pregnancy [26, 81].

Fondaparinux − during its use 10% of anti-Xa activity in mother's plasma was found in umbilical blood; although there were no fetal complications, it is still early to conclude that fondaparinux is safe for use in pregnancy [9, 26, 45, 72]. It has been used in pregnant patients with HIT [71].

Graduated elastic compression stockings (GCS) is recommended when LMWH is contraindicated, or in combination with LMWH after Caesarean section in the presence of several risk factors, or in pregnant women travelling by air longer than 4 h [59, 82]. Using GCS also reduces the risk of postthrombotic syndrome [2].

During lactation warfarin, LMWH, UFH, danaparoid and hirudin are allowed; pentasacharides (fondaparinx) are not recommended [26].

Recommendations for venous thromboembolism prophylaxis in pregnancy and puerperium

In order to diminish the risk of VTE in pregnancy/puerperium all women should undergo assessment of the risk factors for VTE in early pregnancy or, ideally, before pregnancy [6, 18, 45, 83]. This assessment should be repeated during pregnancy, before and after the labor. Based on this assessment an thromboprophylactic plan should be made with haemathologist or other experts if needed [2, 4, 26, 45].

Patients with very high VTE risk are those with previous VTE on the long term warfarin/AT III deficiency/APLA with previous VTE. For them, antepartal high dose LMWH and at least 6 weeks postpatral LMWH or warfarin are recommended [45].

Women with previous recurrent or unprovoked or estrogen provoked VTE, previous VTE with thrombophilia or with family history of VTE and those with asymptomatic thrombophilia (homozygous for FVL or prothrombin gene mutation) with positive VTE family history, are in high VTE risk. For them antepartal and 6 weeks postpartal LMWH prophylaxis is recommended [45].

Women with intermediate VTE risk are those with single previous VTE provoked by transient risk factor no longer present, without thrombophilia, family history, or other risk factors and those with mild asymptomatic thrombophilia or with medical comorbidities, or BMI over 40 kg/m^2. In this group consider antepartal LMWH prophylaxis and apply 7 days−6 weeks postpartal prophylaxis [45].

Lower-risk group consists of women older than 35 years, with BMI higher than 30 kg/m^2, or systemic infection, or OHSS, preeclampsia, ART, immobility, varicous veins, multiple pregnancies, operative delivery, postpartal blood loss more than 1,000 mL. If there is a combination of more than three of those risk factors antepartum and more than two postpartum, LMWH prophylaxis is given antepartum and at least 7 days postpartum [45]. If there is less than three factors ante- and two postpartum, early mobilization and rehidratation are sufficient [45].

Recommendaions for thromboprophylaxis in pregnancy/puerperium according to risk assessment are summarised in the Table 3.

We should emphasize that those recommendations are not meant to dictate the definite course of management; they should, of course, be adjusted to individual patient.

Conclusion

Venous thromboembolism although relatively rare in pregnancy, is a serious problem with hard consequences, so it deserves and demands medical attention. The fact that recognizing risk factors and performing adequate prophylaxis significantly reduces the incidence of venous thromboembolism, obligates us to an active relation towards this problem.

Table 3

Thromboprophylaxis in pregnancy/puerperium according to risk assessment

Risk degree	Risk assessment	Thromboprophylaxis	
	Risk factors	antepartal	postpartal
Very high	- recurrent VTE + AT III deficiency or APLA	high dose LMWH	at least 6 weeks high dose LMWH/warfarin
High	- previous VTE (unprovoked or recurrent or idiopathic or estrogen-provoked) + documented thrombophilia or positive family history of VTE or other risk factors	prophylactic or intermediate dose LMWH	6 weeks prophylactic dose or intermediate dose LMWH
	- asymptomatic thrombophilia (homozygous for FVL or homozygous for prothtrombin gene mutation or combined defects) + positive family history of VTE		

Intermediate	- previous VTE (provoked by a transitient risk factor no longer present, without other risk factors) - asymptomatic thrombophilia (other than those mentioned above) - medical comorbidities (heart/lung /sickle cell/inflamatory disease/SLE / cancer/nephrotic sy/surgical procedure/BMI > 40 kg/m^2)	consider prophylactic dose LMWH (not routinely)	7 days to 6 weeks prophylactic dose LMWH
Lower risk	- age > 35 years - BMI > 30 kg/m^2 - parity > 3 - gross varicose veins - immobility - preeclampsia - dehydratation/hyperemesis - OHSS / ART - multiple pregnancy - smoker - current infection - postnatal: - Caesarean section, - prolonged labour - postpartal haemorrhage > 1 L or blood transfusion	- with more than 3 risk factors: consider LMWH prophylaxis dose - with less than 3 risk factors: mobilization and rehydratation	- with 2 or more risk factors: at least 7 days prophylactic dose LMWH - with less than 2 risk factors: mobilization and rehydratation

VTE − venous thromboembolism; AT III – antithrombin III; APLA – antiphospholipid-antibody syndrome; LMWH – low molecular weight heparin; FVL − Factor V Leiden; SLE – systemic lupus erythematosus; BMI – body mass index; OHSS – ovarian hyperstimulation syndrome; ART – assisted reproductive treatment.

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Terms of clinical research consent's validity

Uslovi za punovažnost pristanka na klinička ispitivanja

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Introduction

Clinical researches of new medicaments and medical products on animals and humans are preceding and unavoidable stage before its application. While from the legal standpoint animals are objects and, therefore, can be considered as things within a clinical research, a human becomes legal subject in all areas of his actions by the moment of birth, including clinical research performed over it. Although underlining this fact might be considered superfluous, World War II and Nazi camps should not be forgotten [1] for the cruelest medical experiments performed against humans without their consent, treating them as mere objects. Modern history also gives examples of humans' abuse for these purposes [2], due to which commenced legal stipulation of the position of people participating in clinical research, as well as terms under which this is possible. Although general agreement exists that clinical trials cannot be denied regardless the risks they carry against health of its subjects – there is also a general agreement that we have to renounce trials performed disrespecting subjectivity of those who are subjects of it [3].

To respect subjectivity of persons who are the subjects of clinical research means their consent to submit themselves to research [4]. We can, thus, name consent the border line between a human as a subject and an object of the research. Realizing its importance, experts from the area of medicine, ethics and law defined terms for consent to be fulfilled in order to consider it valid [5]. Therefore, many international and national regulations set rules for that. However, those regulations are often not harmonized, and the same issue is stipulated in different manners and no clear guidelines are given to researchers how to act, although they are often personally tasked to get subjects' consent. Researchers also do not have all the rules in one document which if to be applied guarantees getting valid subjects' consent and protection of their subjectivity, but also protection from potential researcher's accountability for getting invalid consent.

Having all the mentioned in mind, we collected and analyzed terms that have to be fulfilled in order to consider validity of subject's consent for medical research, starting from solutions in the Serbian law, as well as in international legal and ethics-related documents. In that spirit, we stress as the most important that consent originates from legally competent person or legally authorized representative of legally incompetent person or person incapable of giving consent; it has to be given voluntarily, i.e. must not have shortcomings in a form of the so-called "defects of will"; and must have the attribute of an informed one. A precise definition of terms for validity of research subject's consent in Serbia, however, is not an easy task both for a lawyer and for a physician, since regulations regarding this are given in several legal texts: the Health Care Act [6], the Medicines and Medical Devices Act [7], the Family Act [8], the Obligations Act [9], as well as in Guidelines for Good Practice in Clinical Research (Guidelines) [10], and in international conventions ratified by Serbia. The number of these regulations that have to be kept in mind, can be a problem for a researcher, therefore, objective of our paper was to facilitate researchers in their focus on medical aspects by analysis of legal aspects of consent to clinical researches. Finally, in the last part of the paper, we will indicate also the form of possible accountability of researchers for performing clinical trials with no valid subjects' consent.

Legal competence of the subject

Minimal quality of each consent marking it as "absolutely essential", the term for any experiment against humans, ever since the Nuremberg Code [11] till today, is to be voluntary. Since consent to participate in clinical research in legal terms represents expression of will, it can be considered

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voluntary only if it originates from a person that can formulate legally relevant will, i.e. legally competent person. Legal competency of a person by the rule means its reasoning capability [12–14]. Legally competent (research) subject can be temporarily incapable of reasoning (under influence of alcohol, drugs, etc.), but that is obvious for a researcher and therefore consent getting in such shape can be avoided. However, in some states reasoning capability in this context is considered more relevant than legal competency [15]; thus, it is taken that each person capable of reasoning can give valid consent. Although this position is not illogical, we think that a subject in Serbia has to be legally competent in order to give valid consent (all until corresponding regulations do not foresee differently), and that a researcher has to take care also of the existence of subjects' capability to reason and to inform a competent authority if it recognizes that this lacks temporarily or permanently for some reason.

1. Legal competency is gained by the majority that in different states means different ages – most often 18 (also relevant for Serbia), 21 or 25, with the tendency to lower this limit. Exclusively, legal competency can be acquired even earlier by the so-called public emancipation, if that is acknowledged to a certain person by the decision of competent authority (unlike private emancipation, given by parents, that is not possible in Serbia). Reasons to acknowledge legal competency before the majority are different, and in Serbia that is possible for persons married by the permission of the court or who became parents independently of being married even if in the age of 16 but mature, capable to independently take care of him(her)self and personal rights and interests (Article 11 and 23 of the Family Act). These persons keep their legal competency even if the marriage is terminated. For a researcher it is relevant to obtain legally relevant statement of will from every legally competent person, and therefore also valid consent – independently of how and when the subject gained that legal competency.

Legally incompetent minor, therefore, cannot give duly valid consent to participate in clinical researches, but most of international and national regulations provide an option for that – to be done by their legal representatives instead (or beside them). Serbian Medicines and Medical Devices Act (Article 63) went, however, one step further, prescribing the ban of participation in clinical researches for this category of persons. Exclusively, they can be research subjects with the consent of their legally authorized representatives only if suffering of illness or are in the stages of illness for which clinically tested medicine is intended to (if that is necessary and under special precautions measures); or they are healthy and their participation in the medical experiment is for their interest. Thus, no difference is made between therapeutic and non-therapeutic experiments, as it is done in some European countries [16].

This Serbian regulation, however, does not deal with legally competent or legally incompetent persons, but introduces this ban for persons under 18. Since some minors, as mentioned, can gain legal competency even before the majority, the question is raised are they capable of giving duly valid consent? We could solve this dilemma starting with the question: is getting age of 18 in life legally or medically relevant? The answer is simple: turning 18 is legally relevant because it leads to legal ages and legal competency. From the medical viewpoint, this fact is not relevant, since it happens that exact participation of these persons in research is needed. It is also the fact that different states link legal competency to different ages; and from this viewpoint it is clear that a Serbian legislator obviously had in mind legal competency – not ages as such – when prescribing ban of participation in research for persons under 18. The law linked ban to ages most probably because the number of situations when minors are also legally competent is negligible small. Therefore, it was more correct to use the term "legal competency" in this law instead of indicating ages, as done in for ex. the Health Protection Act (Article 38) regulating validity of the consent to medical measure. We consider that persons under 18 in Serbia can also give duly valid consent to clinical researches if they are legally competent (legal competence means also capability of reasoning, since it is granted by the court. In any case, in order to remove any possible perplexities, the Medicines and Medical Devices Act could also foresee, as by the model of Serbian Organs Transplantation Act [17], that person giving its consent to participate in clinical trails – exactly as person giving consent to donate its organs [18] has to be both legally competent and capable of reasoning, as well of legal age (Article 42 etc.). Although, by the general rule, mentioned characteristics of one person exit's simultaneously (an adult is both legally competent and capable of reasoning) such regulation would solve dilemmas in explicit situations where that is not the case.

The question that is raised in relation to legally authorized representative in this context is: should we make a difference there between persons under 14 and those between 14 and 18 (junior and senior minors), as by general rules of Serbian law contained in the Family Act or not? According to these rules, the difference between these categories of persons are that senior minors are limited in their legal competency and they can independently give duly valid statements of will, and thus also consents to researches – if obtain previous or latter consent of the legally authorized representative. Junior minors cannot independently give statements of will (except for jobs of smaller significance or jobs not creating particular obligations for them), but representatives give consent on their behalf (Articles 64, 72, etc. of the Family Act) – something researcher has to take care of. It is an opinion of ours that these general rules should be valid also in the context of consents to participate clinical researches, because consent is a statement of will as any other.

2. Researchers have to have in mind that not all adults are legally competent. Some of them – incapable of reasoning or to take care of themselves and protection of their rights and interests, or they directly endanger their or others interests (ill persons, person with difficulties in psychophysical development, etc. - Articles 146–147 of the Family Act), can be denied of legal competency totally or partly by the decision of competent authority. These persons also cannot give duly valid consent to clinical research; therefore, a researcher has to inquire do they also have legal competency,

except of being over 18, as requested by the legislator. It would be good to foresee this by regulations, i.e. have this question as a mandatory content of the interview between researcher and potential subject. This is also an argument supporting the position that existence of legal competency is important both for persons over 18 – not only ages. Even if subjects are adults lacking legal competency, their consent given in the so-called *lucida intervalla* of a mental illness, cannot be considered duly valid for legal certainty reasons – until legal competency is acknowledged back by the decision of a competent authority.

According to the majority of international and national regulations, as well as according to the Serbian Medicines and Medical Devices Act (Article 61) both legally incompetent and adults with limited legal competency can be involved in the research, if consent is given by their legally authorized representatives (also including some other terms fulfilled, but not elaborated here since those are not the topic of our paper). By this Act, however, a consent of a legal representative is necessary also for legally competent adults incapable of giving the consent (ex. state of unconsciousness) – under the condition they did not reject to give consent to participate the study before their incompetence begun (Article 66). Mentioned expansion of the group of adults for who consent is given by legal representatives is acceptable, because those persons at the given moment are obviously incapable to give valid consent although formally legal competence exists; however, the question is raised: Who are their legally authorized representatives [19]? Since the mentioned Act does not define this, a representative of the legally competent person in such state cannot be nominated by the researcher – the only solution left is that this should be done by the guardianship body. But, it can perform such action just after it denies legal competency of such person by the procedure prescribed by the Family Act, and this requires time. On the other hand, the question is will then the consent of (newly) appointed guardian be of any relevance, particularly if subject incompetence was of temporary character and clinical research referred exactly to the state in which subject person was (ex. state of unconsciousness). Therefore, we consider that this Act should foresee options of analogue enforcement of regulations from the Health Care Act (Article 34) by which such consent to a medical measure can be substituted by the conclusion of a consilium; however, this option, to our opinion, should be limited only to experiments having therapeutic character.

3. As the following question we should consider: on the basis of which criteria can legally authorized representatives give consent on behalf of the subject they represent [20, 21]? Do they give consent starting from their personal position; i.e. would they give it if they were in the position of the represented person; or they should give it independently of their beliefs (religious and others), but taking care exclusively of interests of the represented person [22]? The second approach is obviously more correct, but what guarantees we may have that representative will act that way? Therefore, it is our opinion that each legally authorized representative or at least guardian as legal representative should obtain consent of the guardianship body, before it gives consent to participation of the represented person in a medical experiment, particularly if it is of nontherapeutic character. In the Family Act of Serbia there already is a provision (Article 137) by which a guardian can give consent to a medical treatment over a protégé only with the consent of guardianship authority; and the Health Care Act (Article 35) foresees the obligations of a health worker to inform a guardian authority if he/she considers that consent of a legal representative (both guardian and parents) to a medical measure is not in the best interest of the represented patient. Since medical experiments are not of the same relevance for the subject, as medical treatment for the purpose of curing, even more important would be to oblige the legally authorized representative to obtain consent of the guardianship authority before giving any further consent.

Hence, the fact is that neither international regulations nor the Medicines and Medical Devices Act. of Serbia which in details regulates clinical research, foresee this – we consider this an omission to be corrected. The literature contains the position by which request to engage guardianship authority needs to be rejected, since it can lead to prolongations of the research commence. Our opinion, however, is that if one subject decides of the submission of other subject to testing which are not medically necessary with certain risks against the subject (which always exist) – then the research commence has to wait and an opportunity has to be given to the guardianship authority to examine the whole situation. To the objection that a guardianship authority is medically incompetent and that ethical committees are sufficient, we have to respond that analogue to that legally authorized representatives are incompetent in this sense, too, as well as subjects, but again asked for their consent. Finally, ethical committees assess permissibility of the clinical research wider, from the aspect of numerous ethical and medical principles; therefore, those cannot sufficiently focus on issues if a legally authorized representative gives a consent justifiably or unjustifiably on behalf of the represented person. Their starting point is a consent as already given and they consider it in other context – was it given on the basis of correctly composed questionnaire, were the subject person and his representative sufficiently informed, etc. (Articles 64 and 73 of the Medicines and Medical Devices Act). On the other hand, guardianship authority has different assessment methods and criteria, other composition and experience in work, as well findings on the relation between the represented person and the representative; therefore, their previous assessment in this domain is considered important, besides the assessment done by ethical committees in a certain latter stage. Finally, if participation in researches can, by the opinion of experts, positively reflect upon the health of legally incompetent subject or it is in his/her interest for other reasons, it should not be suspected that position of his guardianship authority will be positive. The position of the guardianship authority will not be as such only if participation in researches is really problematic from the aspect of represented person and its representative for some reasons intends to give its consent, particularly when the represented person is opposing to that.

The following question imposes this: can a legally authorized representative give consent for participation of a represented person in a clinical research if such person is opposing to it, even after the assessment that it is in his interest? Reasons for this can be plain ones – ex. fear of a child from "people in white", etc. The Medicines and Medical Devices Act gives an answer to this question: consent is acceptable only if it is an expression of assumed will of legally incompetent persons (Article 64 and 66). Therefore, *argumentum a contrario*, their discontent cannot lead to duly valid consent of the legal representative. Regulations of certain states [23], as well some international regulations [24, 25], explicitly foresee that besides the consent of a legal representative, consent of a minor is also necessary; and that the rejection of participation in researches by such subject has to be respected. Exception is allowed if out of research no therapy exists for minor's illness, or minor's therapeutic benefit is in prospect. If elder children are opposing to research, the researcher needs to obtain a license for continuation of an experiment from scientific or ethical committees. Finally, by the Serbian Family Act (Article 62) and the Health Care Act (Article 35), a minor who turned 15 (and capable of reasoning) can individually give consent to a medical measure, and other legally incompetent persons should be involved in the process of decision making related to its undertaking – therefore, there is no reason not to take into consideration the opinion of those persons also in relation to participation in clinical researches.

Voluntary consent

Ban to subject someone to a clinical research or experiment without its free-will consent is foreseen by international [26–28], as well as national legal acts (commonly, by constitutions) and means respect of individual's autonomy [29] and right of every human being to self-determination [30]. The Constitution of Serbia [31] guarantees that "no one can be ….. subjected to medical or scientific experiments without his consent given by free will" (Article 25). This means that expression of will, representing the consent of the subject, must not have any shortcomings, i.e. there has to be harmony between internal and expressed will of the subject. Causes of the disharmony can be numerous.

1. Consent of the subject or his legal representative can be a consequence of fraud, threat or coercion by a researcher or a third person, and therefore it is neither free will nor valid. That originates both from the Guideline, forbidding coercion or other inappropriate impact on the subject (point 4.8.3.); also, from the analogue enforcement of the Serbian Obligations Act referring to expression of will in contracts (Ariticle 60–65, 112 and 117). Such expression of will can be made void, since it is not in harmony with the real expression of subject's will, while person obtaining consent in this way shall also be responsible for that. Hence, it is important to stress that this is right, but not also an obligation of the subject, and therefore its implementation is not obligatory (Article 112 of the Obligations Act).

2. Threat against the subject, however, may not always be an explicit one. It happens that some subjects who are in a dependent relationship with the researcher give their consent under the impact of fear from negative consequences of rejection – although not directly threatened by the researcher. Those, for example, can be medicine students or clinical staff to whom the researcher is a superior, as well as persons whose physician he/she is. In order to prevent this situation, it would be good to exclude the researcher from the process of all or at least such subjects' recruitment; and, this task should be given to an appropriately qualified individual independent on this relationship. Since this is not an easily feasible requirement in countries having low research resources, the other option is to eliminate the mentioned subjects from the list of possible subjects. The Serbian Medicines and Medical Devices Act (Article 63) selected the second option, foreseeing that persons whose free-will consent to participate in clinical research can be influenced by coercion or some other way of impact – cannot be participants of those. The Guideline (point 1.61.) classify this category of subjects as vulnerable subjects, i.e. those for who it is presumed that have diminished ability to protect one's interests manifested by a compromised capability to give voluntary consent to participation in an experiment [32]. Beside them, it is possible also that consent of other categories of vulnerable subjects is not essentially free will due to fear of negative consequences of rejection, and that should be taken care of [33].

3. The right to annul consent belongs also to the subject giving it in deception, where he got under no influence of others, personal or others guilt. Although convinced that he understood well all in relation to forthcoming research, it may happen that the subject got incorrect idea of the real situation and as such gave the consent which for this is not duly valid. This and the next quality of the consent are closely related, since one of the aims of subject's informing before the consent is to be given is exactly to remove any possible deceptions.

4. Finally, consent has to be given seriously, not in a joke, and this is guaranteed also by the requirement to have it formally, i.e. in written, signed and dated, i.e. given before a witness if the subject is not able to read (points 4.8.8. and 4.8.9. of the Guideline and Article 61 of the Medicines and Medical Devices Act).

Informing the subject before giving the consent

The next term of consent fulfillment validity which is required by all international and national regulations, is that a subject has to be informed about what he/she actually accepts prior to the clinical trial participation. This consent is in short named "informed consent". Some regulations, such are international conventions dealing with the problem of human rights, stay at the stage of proclamation – not entering into the details when it is actually considered that this term is fulfilled [Convention on Human Rights and Biomedicine (Article 16); Universal Declaration on Bioethics and Human Rights UNESCO (Article 6), etc.]. International and national regulations of clinical research involving human subjects,

however, elaborate this issue in details. On the basis of the analysis, we conclude that a subject can be considered informed if two conditions are cumulatively fulfilled: one of objective and the other one of subjective character. Objective condition is fulfilled if the subject is provided with all information necessary to overview the situation where he will get into and by this make a decision of consent. Subjective condition means that the obtained information is well-understood by the subject.

Objective condition

It is very obvious in international regulations related to clinical research involving human subjects that different manners that regulate what the subject has to be informed about and in what scope in order to consider his/her consent informed. Some of those determine the scope and the object of informing in an abstract manner, while others concretely list types of information the researcher has to present to the subject [34].

By the Nuremberg Code, the subject can give a consent which is considered informed if he/she sufficiently knows of "the nature, duration and purpose of the experiment, the method and means by which it is to be conducted; all inconveniences and hazards reasonable to be expected; and the effects upon his/her health or person which may possibly come from his/her participation in the experiment" and if he/she understood provided information.

The Declaration of Helsinki stresses the need for subject's adequate informing, necessity of provided information understanding and provided full freedom when consent is being given (Article 24). The object of informing is set a bit wider in this Declaration than in the Nuremberg Code and can purport duty to inform the subject about: "aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study".

The Directive 20/2001 of the European Parliament and the Council from 2001 [35] underlines that the requirement for informed consent is fulfilled if the subject was duly informed on the "nature, significance, implications and risks of the study" [Article 2, par. 1, point (j)].

According to the International Ethical Guidelines for Biomedical Research Involving Human Subjects, informed consent exists if it was given by the competent subject who adequately understood necessary information which was provided (Guideline 4). This document differs from the previously mentioned because it in details regulates what is necessary information that a researcher has to present to a potential subject, giving list of 26 points.

The guidelines for Good Clinical Practice [36] do not define subject's informing as a short-term act, but as "a process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate." As the previously mentioned document, the Guidelines also do not just abstractly determine the scope and the content of the right to be informed (point 4.8.10.). Those elaborate the subject of informing also through indication of a wide list of 20 information to be presented to the subject.

On the basis of the presented we could, above all, conclude that there is a disharmony in respect of terminology used in international regulations to determine scope and object of the obligation to inform the subject, as well as that quite a few regulations define this obligation more concretely. The obligation to inform is mostly defined abstractly and requires more precise definition; this is obviously left to a person obtaining the consent. At the end, this can lead to selective and by subjective criteria chosen scope and object of subject's informing. The space left for assessment in each individual case with how much information the level of "adequate", "necessary" or "sufficient" was reached can endanger reaching the quality for the informed one and by this duly valid subject's consent reflecting negatively in respect of his rights. On the other side, such regulations neither do sufficiently protect interest of researchers who can be treated as responsible for infringement of the obligation to inform the subject.

Under the Serbian law, the scope and object of subject's informing were determined within the Health Care Act – if it is about medical experiments (Article 38), and the Medicines and Medical Devices Act – if it is about clinical trials of medicines (Article 2). The first mentioned foresees that a patient over who a medical experiment is performed has to be "sufficiently informed about the sense, goal, procedures, expected results, possible risks, as well as inconvenient accompanying circumstances of the experiment"; the other mentioned defines informed consent as written statement of the subject "which is given voluntarily after duly informing on the nature, significance, consequences and risk to health". The Medicines and Medical Devices Act in its Article 59 refers also to implementation of already analyzed Guidelines. The mentioned indicates that the Health Care Act determines the scope of an obligation to inform the subject slightly narrower than the Medicines and Medical Devices Act (sufficiently informed is less than duly informed), while the scope of information which are object of informing are determined slightly wider.

Having in mind recognized differences, the question is raised: which legal text the researcher should follow performing the duty to inform the subject, as clinical trails are a certain form of medical experiments? Since in the area of clinical researches the Health Care Act has a character of general one (*lex generalis*), and the Medicines and Medical Devices Act of special one (*lex specialis*), the obligation of informing for the purpose of clinical research should be harmonized with the second mentioned which refers to implementation of the Guideline; therefore, a researcher in Serbia has the obligation of duly informing of the subject about all concrete information foreseen in the Guideline. In this way, respect of autonomy of potential participant in clinical research is fully ensured, as well as credibility of the researcher; therefore, we could conclude that the scope and object of the obligation to inform the subject is adequately and widely set in the Serbian law.

Subjective condition

Fulfillment of just analyzed, objective condition of the informed consent, i.e. providing necessary information to a subject – is not enough, if the subject did not understand it. Therefore, most of the referred regulations particularly underline the necessity to fulfill subjective condition, too. Some researches with this topic show, however, that in practice exactly fulfillment of this condition lacks [37–39], and providing consent to access the study is made equal to fulfillment of its objective, but the formal side of its validity – by communication of necessary information with signing of proper form. Obtaining essentially informed consent, however, has to have features of a process comprised of the procedure of information repetition, its additional clarification, responding to questions, etc. – all to the end of cognition that those are clear to the subject (point 4.8.7. of the Guideline). Important way to achieve this goal is to use words and expressions understandable both for the medically "illiterate" subject (point 4.8.6. of the Guideline). Some studies [40] showed that use of certain methods in order to improve understanding of provided information, like multimedia presentations or extension of the form intended to inform the subject, did not give the expected results. On the other hand, extended discussion on the relation researcher-subject proved as the most efficient way to improve understanding, as well as the use test method, i.e. asking for a feedback in order to check was the provided information understood well. Therefore, with the aim to reach legally and ethically acceptable and essentially duly valid consent to include people in clinical research, we suggest implementation of these methods in the procedure of potential subject selection in Serbia.

Responsibility for conducting clinical researches with invalid consent

If the researcher approaches clinical research with no consent of the subject or with his/her consent not fulfilling the mentioned conditions to be considered duly valid – the issue of researcher's responsibility will be raised, and it can be civil, criminal or both [41].

a) Civil responsibility means obligation of the researcher to compensate material and non-material damage suffered by the subject for being subjected to the clinical research without any or duly valid consent, and it is a fault of the researcher. It is an infringement of personality right. The researcher can't insure him(her)self against the responsibility for this kind of damage, for reasonable causes. Mandatory insurance of the subject refers to possible damages he/she could suffer as a consequence of participation in research performed by law (Article 72 of the Medicines and Medical Devices Act and Article 38 of the Health Care Act), but not from the damage suffered due to its involvement in the research with invalid consent.

b) The second form of researchers' responsibility in this situation is a criminal one, that will be raised if his/her action has features of a criminal act under the name "Illegal Conduct of Medical Experiments and Testing of Medicaments", foreseen in many countries by the Penal Code, as in Serbia (Article 252) [42], in the group of criminal offences against human health. A person shall be responsible for it, if: 1) against regulations performs medical and other similar experiment or clinical testing of drugs against people; and 2) clones people or performs experiments with that objective. While ban to cloning has an absolute character, medical and other similar research involving human subjects or clinical researches of drugs can be performed and responsibility of the researcher shall be raised only if done against regulations. The Guideline (point 4.1.3.) explicitly foresee that researcher has to know and respect regulations applicable in this field. Since the fundamental condition foreseen by Serbian and international regulations for medical experiments is the subject consent (which has to have all the features mentioned in this paper to be considered duly valid) – for this criminal offence shall be responsible the researcher who commits this intentionally without such consent. By the law, criminal offence is committed by the final performance of the described action (a research with no duly valid consent, in our case), and it is not necessary to establish did any other consequence appeared [43].

Beside the mentioned criminal offence, a person who provided the consent of a subject (or his legally authorized representative) to do something, not to do something or suffer in clinical research, by use of coercion, force or threat, shall also be responsible for the criminal offence of coercion (Article 135 of the Penal Code).

Conclusion

On the basis of everything presented in the paper we would like to make several conclusions.

Firstly, it is of tremendous importance for researchers to precisely know which terms have to be fulfilled in order to consider consent to clinical researches duly valid: both for possible serious consequences to the autonomy and subjectivity of the subject and its right to self-determination in regard to its body, as well as for prevention of personal responsibility, and, at the bottom line, for successful finalization of planned researches – without these there is no progress in medicine and no survival of the humankind.

Secondly, it is necessary to have clearly and completely defined terms within the national legislation in order to make researchers aware what are the conditions; those also have to be formulated in cooperation with experts from areas of medicine, ethics and law, taking into the account standards set in international regulations in this field.

Thirdly, researchers shall fulfil terms for getting valid consent in the easiest way if these terms are stipulated within one legal document.

Having all in mind, we could note that at least one of the indicated prerequisites was surely fulfilled in Serbia: obviously cooperation with physicians, ethicists and lawyers existed when terms for subject's valid consent were defined; requirements contained in international legal and ethic documents were also taken into account. This is proved by the fact that Serbian medical law today requires fulfilment of wide span of

terms for duly valid consent of the subject when participating clinical research, and content of some of those terms is well-defined both quantitatively and qualitatively (ex. number and sort of information with which subject in Serbia has to be informed). Serbian regulations, however, have two shortcomings, resulting primarily from the omission of lawyers.

First, some of the terms are not fully defined and some have no sufficiently precise definition – this is underlined by the paper and its authors gave also concrete suggestions to amend and make subject regulations more precise (ex. suggestions regarding relation of legal competency, legal age and subjects reasoning capability; regarding the need to get consent of guardianship authorities in certain situations; regarding actions of persons unable to give consent and having no legal representative, etc.).

The second weakness of Serbian regulations related to clinical researches stems from the first one: in order to remove its incompleteness and impreciseness, it is necessary to consult numerous legal texts – this exceeds capacities of any physician–researcher. Therefore, we consider important changes and amendments of the basic law in this field – the Medicines and Medical Devices Act in ways suggested in the paper, in order to gather all rules in Serbia regarding the provision of the subject's consent in one place and by this enable undisturbed performance of researchers.

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CASE REPORTS

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Myeloma multiplex with pulmonary dissemination

Multipli mijelom sa pulmonalnom diseminacijom

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Abstract

Introduction. Multiple myeloma is a hemathological malignancy characterized by the clonal proliferation of plasma cells in the bone the marrow. Extramedullary dissemination of multiple myeloma is uncommon. In several cases only, the multiple myeloma malignant plasma cells had disseminated to the lung parenchyma. **Case report.** We presented a case of multiple myeloma with lung plasmacytoma, in a 79-year-old patient, hospitalized for febrility and infiltrative mass in the right lung. Two months before the patient was admitted, because of developing terminal renal failure, hemodialysis treatment had started three times a week. Since then, the patient was oliguric, but because of febrility and hemoptysis that appeared, at first he was treated with dual antibiotic therapy which resulted in temporary improvement of his general condition, but pleural effusion remained. After thoracocentesis, followed by myelogram, the multiple myeloma diagnosis was established. **Conclusion.** In patients of middle and older age, with general weakness, exhaustion, loss of weight, renal failure which progresses to the end stage rapidly, if symptoms of respiratory tract occur, consider this uncommon disease – extramedullary dissemination of multiple myeloma.

Key words:
multiple myeloma; neoplasm metastasis; lung; plasma cells.

Apstrakt

Uvod. Multipli mijelom je hematološko maligno oboljenje koje se odlikuje klonalnom proliferacijom plazma ćelija u koštanu srž. Ekstramedularna diseminacija multiplog mijeloma izuzetno je retka. U samo nekoliko slučajeva opisana je diseminacija multiplog mijeloma u pluća. **Prikaz bolesnika.** Prikazali smo bolesnika sa multiplim mijelomom plućne lokalizacije, starog 79 godina, koji je hospitalizovan zbog febrilnosti i infiltrativne promene u desnom pluću. Dva meseca pre prijema, zbog razvoja terminalne bubrežne slabosti, započeto je lečenje hemodijalizom, tri puta nedeljno. Od tog perioda bolesnik je bio oliguričan, a zbog pojave febrilnosti i hemoptizija lečen je najpre dvojnom antibiotskom terapijom, na čiju primenu je došlo do prolaznog poboljšanja opšteg stanja, ali bez povlačenja pleuranog izliva. Nakon učinjene torakocenteze, a potom i mijelograma, postavljena je dijagnoza multiplog mijeloma. **Zaključak.** Kod bolesnika srednjeg i starijeg životnog doba uz opštu slabost, malaksalost, gubitak telesne mase, bubrežnu insuficijenciju koja brzo progredira do terminalne, ukoliko se pojave simptomi u respiratornom traktu, diferencijalno dijagnostički treba razmišljati i o ekstramedularnoj, plućnoj diseminaciji multiplog mijeloma.

Ključne reči:
multipli mijelom; neoplazme, diseminacija; pluća; plazma ćelije.

Introduction

Multiple myeloma (MM) is a plasmaproliferative disease that is most often characterized with uncontrolled monoclonal proliferation of plasma cells in the bone marrow. As a consequence of tumor activity and its products, there are osteolytic lesions, osteopenia with pathologic fractures, followed with hypercalcemia, renal failure and hyperviscous syndrome [1, 2]. It appears in adults, more often male, the average age around 65 years and constitutes approximately 1% of all malignant diseases and slightly more than 10% of all hematologic malignancies [3–5]. The annual incidence in America is approximately 4−5 in 100,000 people and the similar trend is recorded in Europe, too [5–7].

Extramedullary plasmacytoma (EMP) represents approximately 3% of plasma cell neoplasms. EMP are uncommon and typically manifested like solitary plasmocytoma, and about 80% is in the upper respiratory tract, and less than

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5% of all extramedullary plasmocitomas is localized intrapulmonary [8]. Having in mind rates of occurrences of the abovesaid diseases, MM with pulmonary localization is considered as very uncommon.

Renal failure appears in this disease in approximately 50% of patients and is manifested as chronic, but rarely as acute renal failure. Whereas, it is a well-known fact that myeloma spreading to kidneys is an adverse prognostic sign [9, 10].

Chejfec et al. [11] defined even in 1983 the term "myeloma lung" with diffuse infiltrative plasma cells in pleural punctate or tissue samples obtained with needle biopsy and in rarely described cases, with MM spreading to lungs. The diagnosis is often established with biopsy, intraoperatively or by autopsy [11, 12].

Case report

A 79-year old male patient was admitted to our department as a dialysis patient with temporary vascular access (CVC-right jugular vein), pronounced anemic syndrome, febrility and infiltrative mass in the right lung. Regular hemodialysis, three times a week lasting 4 hours, had started 2 months before he was hospitalized in our hospital, but additional difficulties had occurred 4 weeks before he was admitted with body temperature of up to 40°C. He was hospitalized in a regional hospital on suspicion of pleuropneumonia, and dual antibiotic therapy was introduced. This therapy led to lowering body temperature, but the increased levels of acute infection phase reactants remained with sedimentation rate 84 mm/h and C-reactive protein 103 mg/L. According to objective findings, the patient was markedly pale, with visible mucosa and with weakened respiratory murmur in the right lung, with a mass of inspirium crackles and inaudible breathing on the basis, left lung. The radiography of the lungs showed the infiltrative mass in the right lung with the presence of pleural effusion in both, more in the left (Figure 1), while the bronchoscopic findings were normal. The ultrasound of abdomen was almost normal, except the left kidney which was with a larger number of cysts, and the largest one was around 45 mm.

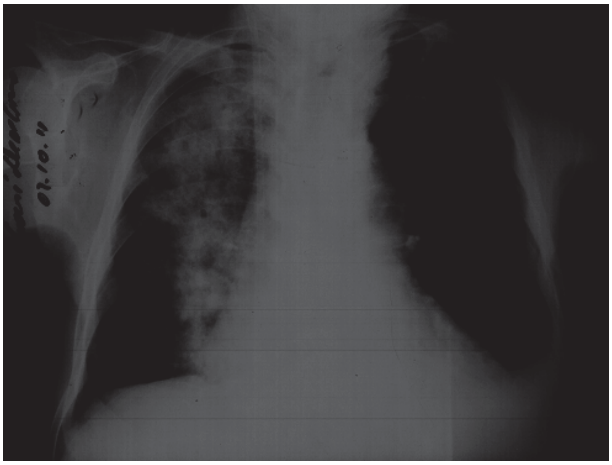

Fig. 1 − Infiltrative mass in the right lung, and the presence of pleural effusion in the left one.

Computed tomography (CT) of the chest confirmed the existing pleural effusion in both lungs and in the right, the zones that would most likely correspond to pneumonitis. Additionally, at the level of the thoracic vertebral body Th9-Th10, there was a hyperdense, solid mass with the diameter of 32 × 19.5 mm, while the other was with the diameter of up to 18 mm at the level of transversal L4 (Figure 2). Osteolytic changes were not observed.

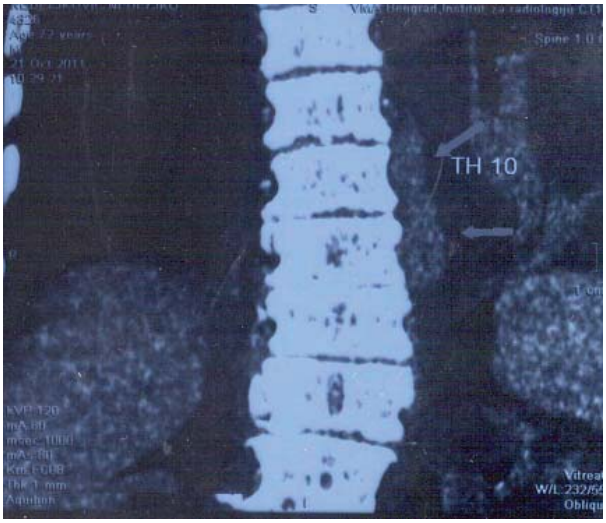

Fig. 2 − Magnetic resonance imaging – a paravertebral tumor mass.

Magnetic resonance imaging (MRI) of the thoracic and lumbar spine confirmed the existence of paravertebral tumor mass on both sides, without any sign of bone destruction.

Laboratory analyses showed anemic syndrome which required substitution (red blood cells 2.87 − 3.33 × 10^{12}, hemoglobin 83−101 g/L, hematocrit 0.26−0.29), while the differential blood test found monocytosis (16−17.3% Mo) with white blood cells 14.9−9.31 × 10^9. The values of urea and serum creatinine were such that the patient was on regular hemodialysis program three times a week (creatinine around 700 μmol/L and urea 20 mmol/L; creatinine clearance rate was of around 6 mL/min). The patient was anuric. The values of alkaline phosphatase ranged from 317−838−407 IU/L, lactate dehydrogenase 914−843 IU/L and once serum hypercalcemia was recorded to be up to 2.71 mg/dL, while the values of total proteins were from 75−87 g/L with normal and slightly lower albumins (35−31 g/L). The completed serum protein electrophoresis indicated M-peak in gamma globulins (36.2%), albumins 44.1%, α1 globulins 4.9%, α2 globulins 7.8%, β1 globulins 2.4%, β2 globulins 4.6%. Immunoglobulin (Ig) λ light chains were 11.8%, and κ light chains 2.49%. The ratio κ/λ was 0.21. Because of the aforementioned pleural effusion, thoracocentesis was done, but the cytological findings of pleural punctuate corroborated plasmacytic infiltration (Figure 3), while in myelogram done subsequently, all the 3 lineages of hematopoiesis were suppressed with 65% by plasma cell infiltration (Figure).

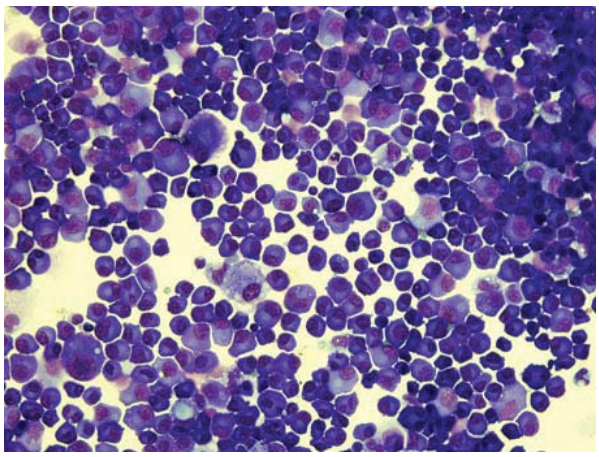

Fig. 3 − Centrifuged deposit of pleural fluid showed plenty of plasmacytoid cells, including binucleated and multinucleated ones admixed with a few reactive mesothelial cells and macrophages (MGG, ×200).

anemic syndrome, and if also spread to the respiratory system, there are symptoms in the upper part of the respiratory tract, too [1–9].

Myeloma cells found at extramedullary site may be because of EMP or due to extramedullar dissemination of multiple myeloma. EMP is an uncommon variant of MM, end it manifests as solitary plasmocytoma. Solitary plasmocytomas occur most commonly in the nasal cavities, paranasal sinuses, nasopharngs lymph nodes, lung, intestinal tract, without bonemarrow involvement. This tipe of plasmocytoma is responsive to local irradiation and has very good prognosis.

Intrapulmonary plasmocytoma is uncommon representing less than 5% of all EMP [8]. In the MM with pulmonary localization there is plasma cells infiltration of the bone marrow and myeloma plasma cells in the lung mass [9]. Several cases of extramedulary plasmocytoma with the involvement of lung parenchyma were only described.

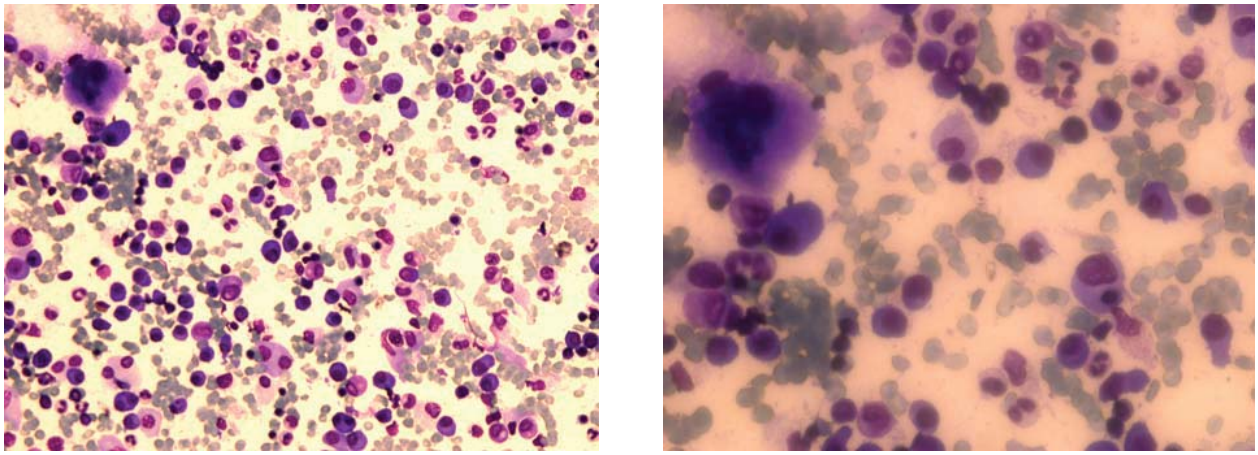

Fig. 4 – Bone marrow examination revealed hypercellular marrow with depression of erythropoiesis and leukopoiesis and adequate megakaryopoiesis, and a large number of plasma cells, above 65% of nucleated cell population, including binucleate and multinucleate forms (MGG: left × 200; right × 400).

Therefore the final diagnosis was multiple myeloma with myelomatous involvment of pleura.

Flat bones radiography, except degenerative changes to pelvis, found no other pathologic changes.

On the account of technical reasons, it was not possible to begin chemotherapy in accordance with the protocol for plasmaproliferative disease so the patient was referred to the competent regional hospital for further treatment. Three days after the patient was transferred, his general condition worsened in terms of his state of consciousness, which was up to the level of somnolence, so that the prescribed therapy was not administered. On the seventh day after his transfer, respiratory arrest appeared which resulted in lethal outcome.

Discussion

In multiple myeloma, the bone marrow is infiltrated with abnormal plasma cells leading to multifocal destructive bone lesions. Clinical presentation of MM is seen in the appearance of general weakness, exhaustion, loss of weight, pain in lumbar spine area, various degrees of renal failure,

In the presented patient, renal failure was the first manifestation of MM, and later pulmonary simptomatology occurred. Because of the infiltrative mass in the lung parenchyma, pulmonary examination was initiated. Computer tomography of the chest showed pleural effusion, but also the tumorous mass paravertebrally bilaterally, while the conducted MRI of the thoracolumbar spine showed no significant changes on bone structures [13–15]. In the course of further examination in order to do diagnostic puncture of pleural effusion, thoracocentesis was done, while cytological findings of pleural punctuate showed numerous plasma cells. Concurrently, laboratory analysis of serum protein electrophoresis showed M-peak in the gamma region [12, 14, 16]. The MM diagnosis was confirmed with biopsy of the bone marrow and the results of more than 65% of plasma cells in myelogram.

Renal failure developed at the very beginning of the disease, while the need for continuous hemodialysis procedures as a sign of irreversible renal failure also corroborated the gravity of the disease and shorter median survival of these patients (on the average 4 months from the first symptoms of the disease).

Dissemination of EMP in the lung is exceptionally rare, and shows up in about 3% of a total number of patients diagnosed with MM [11–13]. Malignant pleural effusion combined with pleural infiltration represents one of the late complications of the disease [17]. The appearance of pleural effusion is an adverse prognostic indicator as well as the resistance to the applied therapy, but also there are great chances for the relapse of the disease despite conducting the radio-and/or polychemotherapy. In some cases, regardless of the therapy, it is also necessary to perform pleurodesis so as to improve the general condition of the patient.

The literature lists the facts that very often, after the appearance of pleural effusion, even with the applied chemotherapy, there is a fatal outcome in the period of less than 4 months.

The presented patient had had the first symptoms of the respiratory tract 3 months before the diagnosis of MM with pulmonary localization was established. In that time, there was a sudden worsening of respiratory symptomatology and progression of pleural effusion so that chemotherapy could not be applied, but the condition resulted in lethal outcome.

Conclusion

Extramedullary dissemination of multiple myeloma in the lung is very uncommon and the prognosis of patients with it is very poor, oppositely to patients with primary pulmonary plasmocytoma to long survival rates. Multiple myeloma is a disease of aged population, with its severe clinical prognosis, heterogeneous symptoms, and the diagnosis is difficult.

Because of that, in patients of middle and older age, with general weakness, exhaustion, loss of weight, anemia, and renal failure, if symptoms of respiratory tract occur, consider this uncommon disease – multiple myeloma with lung involvement.

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CASE REPORT

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Papillary fibroelastoma of the aortic valve

Papilarni fibroelastom aortnog zaliska

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Abstract

Introduction. Primary tumors of the heart are rare, usually benign and occur mostly in adults, and usually originate from the endocardium, followed by the myocardium and rarest of the pericardium. Papillary fibroelastoma accounts for less than 10% of all cardiac tumors, but they are most common valvular tumors. The clinical presentation of papillary fibroelastoma varies from asymptomatic cases to cases which have severe clinical presentation that is most likely due to embolic complications. Tumor can usually be discovered by echocardiography or during autopsy. **Case report.** We reported a case of 53-year-old man submitted to routine echocardiographic examination. The patient had the history of hypertension for five years, without any other symptoms. Echocardiography found a round tumor attached to the noncoronary cuspis of the aortic valve. The tumor was surgically removed and pathohistological examination confirmed diagnosis of papillary fibroelastoma. After surgery the patient fully recovered without tumor recurrence or aortic regurgitation. **Conclusion.** Histologically, papilary fibroelastoma is benign tumor of the heart. As demonstrated in this case, a papillary fibroelastoma can be an incidental finding discovered during echocardiography in patients with the history of hypertension.

Key words:
heart neoplasms; fibroma; aortic valve; diagnosis; echocardiography; histological techniques.

Apstrakt

Uvod. Primarni tumori srca su rijetki, uglavnom benigni i javljaju se najšće kod odraslih. Najčešće ovi tumori potiču od endokarda, ređe od miokarda, a najređe od perikarda. Papilarni fibroelastom čini manje od 10% svih tumora srca, ali ovaj tumor predstavlja najčešći tumor koji se javlja na srčanim valvulama. Klinički, ovaj tumor može biti asimptomatski, ali može i dati teške kliničke simptome koji se uglavnom javljaju zbog embolijskih komplikacija. Ovaj tumor se najčešće otkriva ultrazvukom ili prilikom autopsije. **Prikaz bolesnika.** Prikazan je bolesnik, star 53 godine, koji se liječio pet godina od hipertenzije i kod koga je na rutinskom ultrazvuku otkriven okruglast tumor na nekoronarnom kuspisu aortnog zaliska. Tumor je hirurški odstranjen, a patohistološki je imao strukturu papilarnog fibroelastoma. Nakon hirurškog odstranjenja tumora bolesnik se u potpunosti oporavio i nije imao znakove aortne regurgitacije. **Zaključak.** Papilarni fibroelastom histološki je benigni tumor srca. Ovaj prikaz pokazuje da se papilarni fibroelastom može slučajno otkriti u toku ultrazvučnog pregleda srca kod bolesnika sa hipertenzijom.

Ključne reči:
srce, neoplazme; fibromi; zalistak, aortni; dijagnoza; ehokardiografija; histološke tehnike.

Introduction

Primary tumors of the heart are rare, usually benign, and occur mostly in adults. The prevalence of these tumors ranges from 0.002 to 0.3% in autopsy series. Usually, these tumors originate from the endocardium, followed by the myocardium and the rarest by the pericardium [1–4]. The majority of primary tumors of the heart are benign [4, 5]. Papillary fibroelastoma (PFE) accounts for less than 10% of all cardiac tumors, but it is the most common valvular tumor [6–9]. The clinical presentation of papillary fibroelastoma varies from asymptomatic cases to cases with severe clinical presentation most likely due to embolic complications. Most PFEs are not discovered during echocardiography or autopsy. We presented a case of PFE of aortic valve accidentally discovered during echocardiography.

Case report

A 53-year-old man underwent routine echocardiographic examination without any other symptoms. He had been treated for hypertension five years. Echocardiography found a round tumor with the diameter of 13 mm attached with 6 mm length pedicle to the noncoronary cuspis of the

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aortic valve. The tumor was mobile, round, with echo dense and well-demarcated borders (Figure 1). The tumor did not cause any aortic insufficiency.

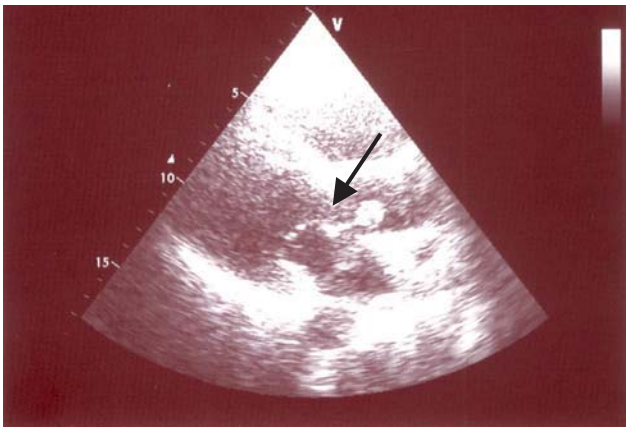

Fig. 1 – Echocardiography (parasternal long axis view) demonstrates a pedunculates mass of 13 mm in size attached to the aortic valve (black arrow).

The patient immediately underwent surgery because of the high risk of embolic complication. The removed tumor was round, soft, grayish, with the diametar of 13 mm. Histopathologic examination showed that the tumor was of papillary configuration. The papillary cord, being an elastic fiber, was surrounded by a mucous layer without blood vessels and covered by a layer of flattened cells. Based on these characteristics, the diagnosis of PFE was made (Figures 2 and 3).

autopsy [10]. The three quarters of hearth tumors are benign. The most common primary benign cardiac tumor is mixoma, while PFEs account for less than 10% of these tumors. The most frequent sites of their occurence are the aortic or mitral valves [11]. In some cases PFE was found in other parts of the heart such as the left ventricle or the atrial septum [12–14].

PFE occurs occasionally, usually in middle-aged and older patients, with a slightly higher incidence in males [2, 15]. Xu et al. [16] described a case of PFE of the tricuspid valve in a 1-mounth-old child. At the time of diagnosis PFE is usually a small tumor with papillary structures of collagen deposits, elastic material and proteoglycans.

To date it remains unclear whether this tumor is true neoplasm, hamartomatous proliferation or organized thromb. Kurup et al. [17] reported that PFE could be associated with irradiation of chest and cardiac surgery. Composition of PFE favors the hypothesis that this tumor represents organized thrombus. Based on the presence of dendritic cells and cytomegalovirus in some patients some authors [18] propose that PFE can be associated with viral endocarditis. De Feo et al. [19] reported a posibility of exposure to environmental pollution with the development of PFE.

Most patients with PFE have no symptoms and the tumors in this patients were incidentally diagnosed on echocardiography, catheterization, cardiac surgery or autopsy [2, 9, 20, 21]. PFE is benign tumor but in some patients torn apart parts of the tumor may lead to embolic complications. Stroke, transient ischemic attack, angina, myocardial infarction or sudden death were the most serious

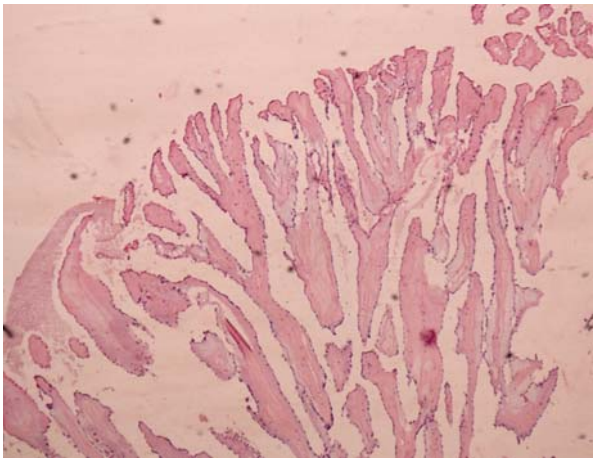

Fig. 2 – The tumor of long, thin papillary structures (HE, × 40).

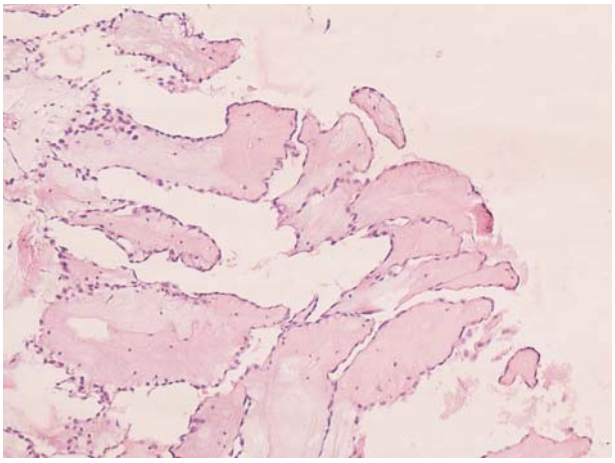

Fig. 3 – The papillary tumor with hyalinized collagenous matrix encountered by uniform single-layer cells without any blood vessel (HE, × 100).

The patient fully recovered after surgery, and echocardiogram 2 and 4 weeks after the surgery demonstrated no tumor recurrence and aortic regurgitation.

Discussion

Tumors of the heart are rare with the prevalence for primary tumors from 0.002% to 0.3% discovered on

complications described, embolism with parts of the tumor, being main reason for the early diagnosis of PFE to be of major interest [6, 15, 22, 23]. Interestingly, valve disfunction is rarely described, although the tumor is usually located on it.

Due to the risk of treatment complication, usually surgical excision of the tumor, it is done in either symptomatic and asymptomatic patients. Patients with tumor size more

than 1 cm, as in the presented case, should be surgically treated by tumor excision due to increased risk of embolization and sudden cardiac death [24, 25]. Asymptomatic patients with nonmobile tumor smaller than 1 cm can be closely followed-up with echocardiography until symptoms develop or tumors enlarges and becomes mobile, and after that tumor could be surgically removed [15, 22]. Patients who from other reasons are not candidates for surgical treatment should be treated with long-term anticoagulation therapy.

Conclusion

Papillary fibroelastomas are histologically rare benign cardiac tumors. Although benign due to their structure and localization these tumors can cause serious embolic complications and sudden cardiac death. Surgical removal of tumors is the best way to prevent possible complications. As demonstrated in this case, papillary fibroelastoma can be an incidental finding in echocardiography.

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CASE REPORT

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Acute psychosis followed by fever – Malignant neuroleptic syndrome or viral encephalitis?

Akutna psihoza praćena febrilnošću – Maligni neuroleptički sindrom ili virusni encefalitis?

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Abstract

Introduction. Neuroleptic malignant syndrome is rare, but potentially fatal idiosyncratic reaction to antipsychotic medications. It is sometimes difficult to diagnose some clinical cases as neuroleptic malignant syndrome and differentiate it from the acute viral encephalitis. **Case report.** We reported a patient diagnosed with acute psychotic reaction which appeared for the first time. The treatment started with typical antipsychotic, which led to febrility. The clinical presentation of the patient was characterised by the signs and symptoms that might have indicated the neuroleptic malignant syndrome as well as central nervous system viral disease. In order to make a detailed diagnosis additional procedures were performed: electroencephalogram, magnetic resonance imaging of the head, lumbar puncture and a serological test of the cerebrospinal fluid. Considering that after the tests viral encephalitis was ruled out and the diagnosis of neuroleptic malignant syndrome made, antipsychotic therapy was immediately stopped. The patient was initially treated with symptomatic therapy and after that with atypical antipsychotic and electroconvulsive therapy, which led to complete recovery. **Conclusion.** We present the difficulties of early diagnosis at the first episode of acute psychotic disorder associated with acute febrile condition. Concerning the differential diagnosis it is necessary to consider both neuroleptic malignant syndrome and viral encephalitis, i.e. it is necessary to make the neuroradiological diagnosis and conduct cerebrospinal fluid analysis and blood test. In neuroleptic malignant syndrome treatment a combined use of electroconvulsive therapy and low doses of atypical antipsychotic are confirmed to be successful.

Key words:
neuroleptic malignant syndrome; encephalitis, viral; diagnosis; diagnosis, differential; treatment outcome.

Apstrakt

Uvod. Maligni neuroleptički sindrom je retka, potencijalno fatalna, idiosinkratska reakcija na antispihotičnu terapiju. U nekim kliničkim slučajevima je teško postaviti diferencijalnu dijagnozu između malignog neuroleptičnog sindroma i akutnog virusnog encefalitisa. **Prikaz bolesnika.** U ovom radu prikazan je bolesnik kod koga je prvi put dijagnostikovana akutna psihotična reakcija i započeto lečenje tipičnim antipsihotikom, nakon čega je došlo do razvoja febrilnosti. Klinička slika odlikovala se znakovima i simptomima koji su mogli ukazivati i na maligni neuroleptični sindrom i na virusno oboljenje centralnog nervnog sistema. U cilju detaljnog dijagnostikovanja urađene su dodatne procedure: elektroencefalografija, magnetna rezonanca glave, lumbalna punkcija i serološke analize likvora. S obzirom na to da je nakon toga isključen virusni encefalitis i postavljena dijagnoza malignog neuroleptičkog sindroma, bolesniku je odmah isključena antipsihotična terapija. Lečen je prvo simptomatskom terapijom, a potom atipičnim antipsihotikom i elektrokonvulzivnom terapijom, nakon čega je došlo do potpunog oporavka. **Zaključak**. Prikazane su teškoće rane dijagnoze kod prve epizode akutnog psihotičnog poremećaja udruženog sa akutno nastalim febrilnim stanjem. Diferencijalnodijagnostički je neophodno razmotriti pojavu malignog neuroleptičkog sindroma i virusnog encefalitisa, odnosno potrebno je uraditi neuroradiološku dijagnostiku, i analizu likvora i krvi. U terapiji malignog neuroleptičkog sindroma potvrđena je uspešnost kombinovane primene elektrokonvulzivne terapije i niskih doza atipičnog antipsihotika.

Ključne reči:
neuroleptički maligni sindrom; encefalitis, virusni; dijagnoza; dijagnoza, diferencijalna; lečenje, ishod.

Introduction

In the psychiatric clinical practice, it is sometimes very difficult to differentiate an organic acute psychotic reaction from neuroleptic malignant syndrome (NMS) [1, 2]. NMS may mask an infectious disease of the central nervous system (CNS), while, on the other hand, CNS infection itself may be a risk factor of faster and easier onset of NMS as a response

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to the applied antipsychotic drug therapy [3, 4]. However, acute viral encephalitis, whose clinical picture primarily includes pronounced psychiatric phenomenology, constitutes a particular problem.

Acute viral encephalitis is a non-purulent inflamatory disease of the brain, most commonly caused by *Herpes simplex* viruses – type 1 (HSV-1) and type 2 (HSV-2), enteroviruses, or arboviruses [4]. The onset is abrupt, acute and followed by high temperature and headache. Disorders of consciousness, personality and mood (particularly in cases of HSV encephalitis), epileptic seizures, hemiparesis, or meningeal signs and symptoms are also common occurances. The most accurate diagnostic tool is brain biopsy, but it is non-applicable. The other reliable parameter is the polymerase chain reaction (PCR) test, with the sensitivity of 95%, and specificity of 100% for *Herpes simplex* viruses. However, the other laboratory values are not considered to be a reliable aid in the diagnosis of viral encephalitis, since the serological test of the cerebrospinal fluid (CSF) very often remains within the normal range.

NMS is a rare (0.02–3.22%), idiosyncratic adverse reaction to antipsychotic drug therapy, and it is not directly associated with antipsychotic drugs dosage [1, 5]. Such idiosyncratic adverse reaction to antipsychotic drug therapy occurs as a result of the polymorphism of dopamine receptors and it is associated with individual genetic vulnerability to antipsychotic medications. All antipsychotics, both typical and atypical, might cause NMS, but high-potency antipsychotic medications (haloperidol, fluphenazine) are considered a more common cause of this syndrome [6]. The etiology of NMS has not been fully elucidated yet; it is thought that blockade of the dopamine transmission in the nigrostriatum causes muscle rigidity, tremor and rhabdomyolysis, while the dopamine blockade in the hypothalamus leads to the changes in thermoregulation and hyperthermia [2]. Death occurs as a result of respiratory depression, cardiovascular collapse, renal dysfunction, rhabdomyolysis, disseminated intravascular coagulation (DIC) or pulmonary embolism. In recent years reduction in mortality caused by NMS has been registered, i.e. until 1970 mortality rate was up to 76%, but since 1980 it has been gradually reducing and now it is 10–20% [7]. Some 90% of patiens the developed NMS recover within 2 to 14 days. This period was prolonged up to 35 days in patients receiving a depot preparation of antipsychotic drug [7].

In all the patients who had high temperature accompanied by the change in mental status, the lumbar puncture was indicated in order to exclude a possible CNS infection. Lumbar puncture and the electroencephalogram (EEG) are essential diagnostic procedures for elucidating and determining the etiology of the altered mental status [8, 9]. EEG is an early and sensitive indicator, so it can be used to help in the differential diagnosis of some cases. During the acute phase of the disease focal abnormalities are often recorded on EEG. The severity of EEG abnormalities does not usually correlate with the severity of the disease (encephalitis), but their improvement predicts good prognosis [10, 11].

Case report

A 22-year-old soldier prior to joining the Army functioned adequatelly in all the spheres of his life. The patient was treated in the regional military hospital, diagnosed with acute psychotic reaction. As soon as his general condition deteriorated (after 13 days), he was referred to the Clinic for the Internal and Emergency Medicine of the Military Medical Academy (MMA), where he was kept for 3 days, and then transferred to the MMA's Psychiatry Clinic for further treatment which lasted 39 days. So, his treatment lasted two months altogether.

Based on the heteroanamnestic data (the mother) and the available medical documentation, it was found out that 10 days prior to his admission to the regional military hospital his mood altered: he was nervous, tense, inclined to conflict situations, physically agressive, complaining of severe headaches and caugh. On admission he was psychomotorically agitated, with paranoid and bizarre sexual delusions. The patient was initially treated with high doses of anxiolytics – diazepam 50 mg/day and on the 11th day since the start of the treatment he was prescribed haloperidol, parenterally, at a total daily dose of 30 mg. As a reaction to this therapy a sudden change in his mood occured (he became negativistic and mutistic). A day after the antypsychotic therapy was administered, his body temperature elevated up to 38°C, and some laboratory values increased: creatine phosphokinase (CPK) – 1706 U/L (normal values 26–200 U/L), leucocytes – 15,000 $\times 10^9$/L (normal 4,000–10,000 $\times 10^9$/L), ALT – 135 U/L (normal 10–49), AST – 73 U/L (normal 0–37 U/L). As for the neurological status rigidity and tremor were registered in all the extremities. As the differential diagnosis NMS and viral encephalitis were considered. Antipsychotic therapy was immediately stopped. Because of the suspected encephalitis (headache, cough, febrility, altered state of consciousness), computed tomography (CT) of the head and lumbar puncture were performed. CT was normal. The lumbar puncture showed 8 lymphocytes/mm^3 (normal values – lymphocytes $\leq$ 5 mm^3). The serological tests of the CSF were normal.

Due to the poor somatic state the patient was transferred (on the day 13) to the Clinic for Internal and Emergency Medicine of the Military Medical Academy. On admission he was conscious, disoriented, poorly communicative, hypertensive (200/120 mmHg), febrile (38.6ºC), with rigidity and tremor reported in his neurological status. The antibiotic (ceftriaxone 2 g/day), antihypertensive and anticholinergic (biperiden 10 mg/day) therapies were immediately started. After the second lumbar puncture analysis of the cerebrospinal fluid showed 5 elements/mm^3 (normal – lymphocytes $\leq$ 5 mm^3), glucose 5.7 mmol/L (normal values 2.2–4.4 mmol/L); other findings from cerebrospinal fluid (CSF) (serological analysis, proteins) were normal. On the day 14 after the initiation of the treatment EEG reading indicated theta dysfunction in the right frontocentral region.

Since the differential diagnosis ruled out viral encephalitis, on the day 15 from the start of the tretament the patient was transferred to the Psychiatry Clinic. Upon the admission

to the Clinic he was febrile (38.6ºC) and very agitated. The blood values obtained by the repeated laboratory tests showed the leucocyte count of 20,000 × 10^9/L, and the erythrocyte sedimentation rate (SE) of 30 mm/1h (normal values < 25 mm/1 h). His blood pressure was elevated (160/110 mmHg). The rigidity in muscles accompanied by the pronounced tremor and excessive sweating was still present in his neurological status. The slowness of movement and speech, disorientation in time and space and confusion were identified by psychiatric observation. In the course of his treatment daily oscillations from somnolency to the state of agitation with paranoid delusions were registered. At that point the patient was treated only with symptomatic therapy (intravenous rehydration polyvitamin, diazepam in high doses – 50 mg/day, antibiotics – ceftriaxone 2 g/day, antihypertensives).

On the day 22 after the start of the treatment the patient's laboratory values and blood pressure were normalized, so his antihypertensive and antibiotics therapy was withdrawn. The rigidity and tremor also disappeared and during the examination of the muscle tone an active muscle resistance was noted. With the improvement of his somatic condition the clinical picture of confusion, delirium syndrome was becoming more pronounced. Therefore, an atypical antypsychotic drug (clozapine 75 mg/day) and an affective stabilizer (valproate 1500 mg/day) were introduced into his therapy on the day 23 from the start of the treatment. Since the above-described clinical picture persisted even after the administered antipsychotic drug therapy, electroconvulsive therapy (ECT) was added to his therapy on the day 28, with the informed consent of the patient's close relative. The course of ECT consisted of 4 applications administered in the 2 following weeks.

EEG was performed 3 times (on the days 14, 21 and 34) and each time theta dysfunction was recorded in the right frontal region. The patient also underwent magnetic resonance imaging (MRI) of the head with contrast – the findings were normal. One month after the beginning of the treatment the patient was clinically stable, with no symptoms of confusion, delirium syndrome, but with persistent paranoid delusions. The therapy was not changed and on the day 48 the fourth EEG was performed, which, in comparison to the previous findings, displayed the phase of recovery. The fifth EEG, carried out at the completion of his hospital treatment (on the day 75), was normal. Medical check-ups in an outpatient setting (the first after 2 weeks and later repeatedly at one-month intervals), showed that the patient was in stable remission, with complete distancing from paranoid delusions. He underwent antipsychotic drug therapy with clozapine and an affective mood stabilizer (valproate) given at the remission-maintaining doses of 50 mg and 1000 mg *per* day respectively. Six months upon the completion of his hospital treatment full recovery with normal clinical psychiatric and neurological findings was recorded. The laboratory values of biochemical and hematological values fell within the normal ranges. The EEG readings were normal. As the patient's medical condition and recovery remained stable, the therapy was withdrawn.

Discussion

NMS most often occurs during the first administration of antipsychotic drug therapy, or after the increase in the dose of antipsychotic. Risk factors associated with the development of neuroleptic malignant syndrome range from the ambient temperature rise, dehidration, agitation or catatonia, the use of high-potency neuroleptic drugs or depot preparations, to an organic brain damage and the concomitant use of lithium or anticholingerics.

To diagnose NMS it is necessary to take a thorough medical history, perform somatic and neurological examination and monitor laboratory parameters [12]. For now, there are no generally accepted diagnostic criteria for NMS, but the most commonly used criteria are DSM IV [13] and Levenson criteria which require the existence of all the 3 major symptoms (fever (> 38ºC), rigidity, increased CPK), or 2 major and 4 out of 7 minor symptoms (tachycardia, abnormal blood pressure, tachypnea, leukocytosis (10,000–40,000 × 10^9/L), altered consciousness, sweating) [7, 14].

The presented patient developed a clinical picture of NMS within 24 h after the administration of the typical antipsychotic, haloperidol, in the daily dose of 30 mg. Information about the occurence of a strong headache and cough in early stages of the disease required a detailed examination of the patient, because the differential diagnose raised the question whether NMS masked the primary CNS infectious disease [3, 4, 14]. For this reason and also because of the lymphocytic pleocytosis (8 limphocytes/mm^3 – on the day 10 from the start of the treatment), the patient was thoroughly neurologically and infectologically examined (lumbar puncture, serological analysis CSF, CT, MRI, EEG).

EEG was performed on several occasions (on the days 14, 21, 34, 48 and 75). Theta dysfunction was registered in the right frontocentral region, corresponding with the period when confusiondelirium syndrome was most prominent (on the days 14, 21 and 34). As the patient's clinical findings improved, the EEG results improved as well (on the day 48 and 75).

Such changes in EEG were also mentioned by other authors who were describing NMS [15]. Very few studies so far performed have established that it is most likely that dopamine blockade leads to changes in neural pathways, causing abnormal EEG [16].

The diagnostic criteria for encephalitis [10, 11] are: general signs of inflammation, including changes on the skin, mucous membrane and lymph nodes; neurological signs (hemiparesis, aphasia, ataxia, pyramidal signs deficits, autonomic dysfunction, etc.); changes in laboratory blood tests (lymphocytosis is characteristic of viral encephalitis, while leucopenia and thrombocytopenia are characteristic of viral hemorrhagic fevers); changes in X-ray of the lungs; changes in EEG readings, most often temporofrontally slow rhytm or recurrent complex [17]; changes in CT or MRI of the head (with contrast), such as hyperintensity, edemas, hemorrhage, or inflamation; temporal lobe hyperperfusion registered on Single Photon Emission Computed Tomography (SPECT); positive findings obtained by lumbar puncture: lymphocytic

pleocytosis (> 5 lymphocytes/mm^3), glucose normal, proteins normal/or slightly elevated. The results of > 5 lymphocytes/mm^3 are found in 95% of acute viral encephalitis cases; serological analysis of CSF.

Table 1 shows that the patient met all the criteria for NMS, while the evidence for acute viral encephalitis was a suspicious CSF finding (borderline lymphocytes *per* mm^3), as well as general signs at the biginning of the treatment (headache, cough). The changes verified in EEG are described in NMS, as well as in acute viral encephalitis [10, 15, 16].

activity in the brain. First effects of ECT therapy are usually registered after the fourth application of ECT [14], which is exactly the number of treatments the respective patient received. The application of ECT should be particularly considered when it comes to patients who do not show any signs of improvement even 48 h after the pharmacological treatment, or when the clinical picture is not clear, i.e. when the cause of the symptoms could be NMS, malignant catatonia, or mood disorder [14].

Table 1

Comparison of diagnostic criteria for neuroleptic malignant syndrome (NMS) and viral encephalitis in the presented case

Diagnostic criteria	Criteria present
Features common to both disorders	
hyperthermia	+
leukocytosis	+
altered consciousness	+
EEG: diffuse slowing and focal abnormalities	+
Distinguishing features	
Features more common to NMS	
elevated CK	+
muscle rigidity	+
tachycardia	+
tachypnea	+
abnormal blood pressure	+
diaphoresis	+
Features more common to viral encephalitis	
general signs of infection, headache, cough	+
focal neurologic signs	-
MRI: focal signs	-
abnormal chest x-ray findings	-
cerebrospinal fluid: pleocytosis	Borderline findings

EEG − electroencephalogram; CK − creatine kinase; MRI − magnetic resonance imaging.

After an episode of NMS it is necessary to include antipsychotics into the therapy again, in order to control psychiatric symptoms. The scientific literature contains evidence that rechallenging the patient with the same antipsychotic results in NMS recurring in 5 out of 6 cases. The use of lower potency antipsychotics is safe in 9 out of 10 cases. The introduction of atypical antipsychotic in a lower dose with regular monitoring for symptoms of NMS is one of the safest methods of treatment [2].

After the withdrawal of NMS symptoms (on the day 23 from the start of the treatment) the patient presented in this case was given clozapine in small doses [2, 18] and ECT was performed, as well.

Electroconvulsive therapy can reduce hyperpyrexia, perspiration, delirium, probably by modulating the dopamine

Conclusion

We presented the difficulties in early diagnosis at the first episode of acute psychotic disorder associated with acute febrile condition. For the differential diagnose it is necessary to consider potentially fatal NMS and viral encephalitis, i.e. neuroradiological diagnostics, cerebrospinal fluid analysis and laboratory tests need to be done. In the treatment of NMS, in addition to excluding the prescribed antipsychotic and administration of a symptomatic therapy, a combination of ECT and low doses of atypical antipsychotic proved to be successful. Outpatient follow-up (6 months after the hospital treatment), alongside with monitoring of laboratory parameters and follow-up EEG, showed a complete recovery of the patient.

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Terminology, diagnostics and therapy of laryngopharyngeal reflux – A glimpse into the past

Terminologija, dijagnostika i terapija laringofarinksnog refluksa – pogled u prošlost

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Key words:
larynx; gastroesophageal reflux; diagnosis, differential; therapeutics; hydrochloric acid; pepsin a.

Ključne reči:
larinks; gastroezofagusni refluks; dijagnoza, diferencijalna; lečenje; hlorovodonična kiselina; pepsin.

Introduction

Laryngopharyngeal reflux (LPR) is the presence of refluxed gastric contents in the laryngopharyngeal space, where it is in close contact with the tissues of upper aerodigestive tract. Some authors consider it an entity, while others as extraesophageal manifestation of gastroesophageal reflux. However, it is considered that its pathophysiology and symptoms are different from gastroesophageal reflux. Main symptoms are: throat clearing, hoarseness, chronic cough, postnasal drip, halitosis, dysphagia, sense of foreign body in pharynx. As a confirmation of suspected LPR, indirect laryngoscopy shows: mucous edema, redness, ventricular obliteration, pseudoulcer, hypertrophy of posterior laryngeal region and laryngeal granulomas. It is believed that the presence of gastric contents in larynx and hypopharynx plays a role in the development of cancer lesions of this region. Today, an influence of pepsin and hydrochlorid acid on many other disorders in otorhinolaryngological region are being considered [1].

At the level of assumptions

Although in the middle of XIX century the only possible way to visualize larynx was *post mortem*, negative impact of numerous factors on different laryngeal functions, predominantly phonation, were known. Singing teacher in Paris, Manuel Garcia [2], wrote "Studies about human voice" in 1840, where he stated: "Gourmet dishes, oils from certain herbs, spirits, it all has negative impact on our apparatus." Thus, he pointed out a possible influence of reflux on larynx, without actually being able to prove it. Guided by a wish to show larynx, in 1854 Garcia performed the first "autolaryngoscopy", and managed to visualize this organ for the first time. By the end of the XIX century, changes of posterior part of larynx were confirmed histopathologically, which caused a series of researches on gastric contents reflux as an etiology factor in many laryngeal disorders in the XX century.

In 1880, Rudolf Virchow wrote a descriptive term "pachydermia verrucosa laryngis" for changes on arytenoid mucosa, which he associated with vocal abuse. In his original paper, Virchow described it as a state different from laryngeal leucoplacia and keratosis [3]. In the same year, Morell Mackenzie [4] identified the changes of arytenoid cartilage based on the presence of ulceration. Journal of the Royal Society of Medicine in 1908 published the abstract of W. Jobson Horne's [5] presentation, in which he confirms that pachydermia is a consequence of hyperplasia due to persistent laryngitis, but he identified rather different causes. In the cuts of a 37-year old patient's vocal cords, besides hyperplasia, he found excrescence with sulcus in front of and behind it.

In 1928, Chevalier Jackson [6] observed 217 patients during a 4-year period, and named changes he found on arytenoids "contact ulcer". It was almost 50 years after Mackenzie's description. Jackson [6] emphasized that this term refers strictly to nontuberculous lesions, but that any of them can turn into cancer. He associated these lesion to excessive vocal use, occurring frequently among salesman, doctors who gave lectures, priests, with the significant influence of alcohol and tobacco use. In his work about laryngeal keratosis in 1946, Clerf [7] also said that pachydermia occurred more

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often at alcoholics or excessive vocal use, as in street sellers, for example.

From posterior laryngitis to "silent" gastroesophageal reflux

The advance in understanding the posterior laryngeal changes happened in 1968, when first reports on posterior laryngitis caused by gastroesophageal reflux occurred. That year Delahunty and Cherry [8] concluded on animal model that described changes on posterior larynx were caused by gastroesophageal reflux, while Cherry and Margulies [9] treated three patients with posterior laryngitis, and changes disappeared in six months. In 1970, Delahunty and Ardran [10] tried to explain globus hystericus as a possible manifestation of reflux esophagitis. In 1972, Delahunty climbed one floor above larynx, and in "Journal of Laryngology" published the paper titled "Acid laryngitis" [11]. In it, he stated as a postulate that "patients with reflux esophagitis can regurgitate acid contents even more, especially during the night, which causes inflammatory reaction of posterior larynx".

Searching PubMed data base showed a lack of papers until 1984, when a research of otorhinolaryngologists from Ljubljana, Vinko Kambič and Zora Radšel [12], "Acid posterior laryngitis" was published in the same journal in which Delahunty published the results of his researches. They published the histological results of biopsy of mucosa in 44 patients with chronic posterior laryngitis caused by acid. From phoniatric point of view, Živko Majdevac [13], student of Czech phoniatric school and founder of Phoniatric Department of Otorhinolaryngology Clinic in Novi Sad, was also interested in these changes. At the VIII Congress of UEP (Union of European Phoniatrics) in 1979 in Köszeg (Hungary), he presented his "Classification of dysphonia regarding primary etiological factor", and in the first group of disorders – functional disorders, described "contact hyperplastic dysphonia" as a condition which occurs in men whose voice is in range of baritone or bas, and more often at military commanders and teachers. During phonation, posterior thirds of vocal cords are overcontracted, which causes the appearance of circumscriptive yellowish thickening. At the time, he associated the mentioned changes on vocal processes of arytenoid cartilage with gastric hyperacidity, considering it a significant cofactor.

In 1986, Benjamin [14] published a paper on extraesophageal complications of gastroesophageal reflux, stating that gastric contents can get from esophagus into hypopharynx and lungs. In 1989, Wilson et al. [15] published a paper based on a 23-hour long ambulatory measurement of pH and biopsy of posterior larynx in patients with hoarseness, throat burn and globus sensation.

In 1991, Olson [16] mentioned laryngopharyngeal manifestations of gastroesophageal disease. In the same year, James A. Koufman [17] published a paper on otolaryngeal manifestations of gastroesophageal disease in 225 patients, which was the highest number of patients up to the time. At that moment, he did not refer to the disorder as LPR yet, but called it "occult (silent) gastroeophageal reflux disease". A paper on measuring pH on children with gastroesophageal reflux was published in 1992 by Contencin et al. [18].

Gastroesophageal *vs* laryngopharyngeal reflux

In 1996 in the Journal of Voice, Koufman, Sataloff and Toohil [19] published a report from consensus conference on LPR, and that was the first paper to refer to the term laryngopharyngeal reflux. In 1992, Kamel et al. [20] published the results of prospective study on the use of omeprazole in the therapy of reflux laryngitis, while Shaw et al. [21] in 1996 published a paper with the special emphasis on laryngoscopic findings before and after the therapy with omeprazole. In 1997, Wo et al. [22] published a work on empiric use of high doses of omeprazole in patients with posterior laryngitis, while Habermann et al. [23] published a study on *ex iuvantibus* therapy with pantoprazole in patients with posterior laryngitis in 1999. In 1999, Koufman [24] made a report about the treatment of LPR (for the first time with this name) on I International Symposium about human pepsin, held at the University of York (England). The term of laryngeal reflux in children was first mentioned in 2000 by Zalzal and Tran [25] in the paper titled "Pediatric gastroesophageal reflux and laryngopharyngeal reflux". The term LPR was not yet used in that year, but "laryngitis gastrica" was used instead.

In 2000, Koufman et al. [26] found that reflux is observed in 50% of their examined patients with laryngeal and vocal problems. In June 2001, Belafsky, Postma and Koufman [27] published a paper in the Laryngoscope Journal, in which they explain their scoring system of reflux findings [*Reflux Finding Score* – (RFS)], questionnaire about the LPR symptoms named Reflux Simptom Index (RSI) and support the term LPR. In August of the same year, in the same journal, they explained the validity and usefulness of RFS in detail [28]. A year later, the same team [29], (joined by Amin) made a detailed evaluation of RSI usefulness, while Koufman [30] definitively explained that LPR is different from classic gastroesophageal reflux disease.

Nissen's fundoplication as a method for the treatment of extraesophageal manifestations of gastroesophageal reflux disease was promoted in the study by Lindstrom et al. [31] in 2002. In 2003 Siupsinskiene and Adamonis [32] published a paper on *ex iuvantibus* diagnosis by administration of omeprazole and monitoring the response. In the same year, Galli et al. [33] published a study based on the hypothesis that biliary reflux, as well as gastric reflux, has an influence on inflammatory, precancerous and neoplastic changes of larynx. The first paper on the quality of life in patients with LPR was published in 2007 [34].

In April 2011, during 132nd annual meeting of American Laryngology Association (ALA), Jamie A. Koufman [35] was awarded for extraordinary contribution to the research and medical references in laryngology. It was a reward for a lifelong work on explaining etiology, pathophysiology, clinical findings, diagnostics, therapy and prevention of laryngopharyngeal reflux. In order to grasp the importance of her contribution, it should be mentioned that among the scien-

tists who got this award were Chevalier Jackson and John Kirchner.

Conclusion

Researches on the influence of laryngopharyngeal reflux on the organs of otorhinolaryngology region keep on, especially after understanding that this disorder can be a stand-alone factor or cofactor in etiology of secretory otitis, chronic rhinitis, nose-sinus polyposis and cancer of aerodigestive ways. However, without looking back in the past and paying tribute to everyone who explained the term and nature of laryngopharyngeal reflux, no new researches would have the full sense.

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