ЗВАНИЧАН ЧАСОПИС СРПСКОГ ЛЕКАРСКОГ ДРУШТВА

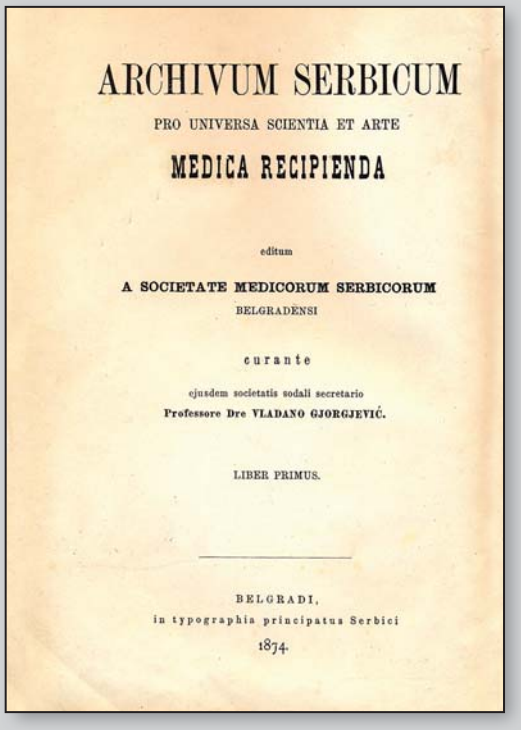
СРПСКИ АРХИВ

ЗА ЦЕЛОКУПНО ЛЕКАРСТВО Осн.1872

ГОДИШТЕ / VOLUME 145 • ЈУЛ–АВГУСТ 2017 / JULY–AUGUST 2017 • СВЕСКА / ISSUE 7–8

OFFICIAL JOURNAL *of* THE SERBIAN MEDICAL SOCIETY, Est.1872

SERBIAN ARCHIVES *of* MEDICINE



ARCHIVUM SERBICUM

PRO UNIVERSA SCIENTIA ET ARTE

MEDICA RECIPIENDA

editum

A SOCIETATE MEDICORUM SERBICORUM

BELGRADENSI

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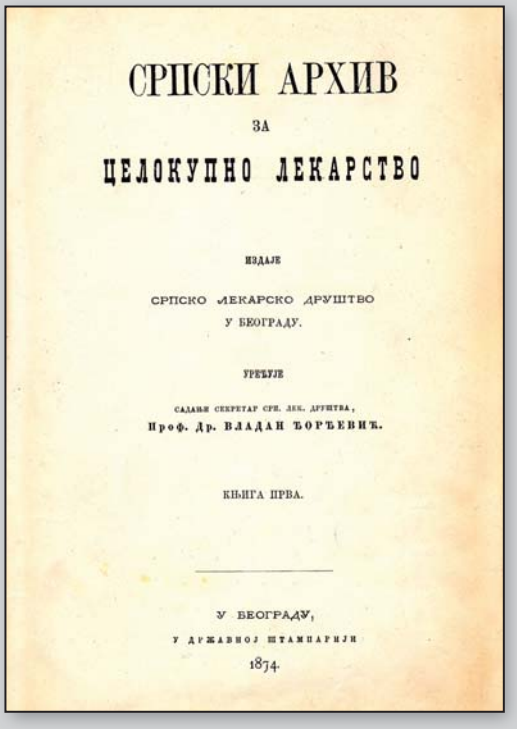

СРПСКИ АРХИВ

ЗА

ЦЕЛОКУПНО ЛЕКАРСТВО

ИЗДАЈЕ

СРПСКО ЛЕКАРСКО ДРУШТВО

У БЕОГРАДУ.

УРЕЂУЈЕ

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1874.

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Српски архив за целокупно лекарство је часопис Српског лекарског друштва основан 1872. године, у којем се објављују радови чланова Српског лекарског друштва, претплатника часописа и чланова других друштава медицинских и сродних струка. Часопис објављује: оригиналне радове, саопштења, приказе болесника, прегледе литературе, актуелне теме, радове из историје медицине, радове за праксу, радове који се односе на језик медицине, радове из медицинске етике (клиничка етика, етика публиковања, регулаторни стандарди у медицини), извештаје с конгреса и стручних састанака, стручне вести, приказе књига и дописе за рубрике Сећање, *In memoriam* и *Promemoria,* као и коментаре и писма Уредништву.

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Srp Arh Celok Lek
ISSN 0370-8179
UDC 61(497.11)
COBISS.SR-ID 3378434
Српски архив за целокупно лекарство
Званичан часопис Српског лекарског друштва
Излази шест пута годишње

Оснивач, власник и издавач
Српско лекарско друштво
Џорџа Вашингтона 19, 11000 Београд, Србија
Председник
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355-1009094-22

Чланци у целости доступни су на интернет страници: www.srpskiarhiv.rs

Цена претплате за календарску годину је 3.000,00 динара за појединце, 6.000,00 динара за установе и 100 евра за читаоце ван Србије. Цена појединачног примерка је 600,00 динара, а свеске из претходних година 300,00 динара.

Штампање „Српског архива за целокупно лекарство" током 2017. године помогло је Министарство просвете, науке и технолошког развоја Републике Србије

ISSN 0370-8179; ISSN Suppl 0354-2793

eISSN 2406-0895
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Штампано у Србији

СРПСКИ АРХИВ
ЗА ЦЕЛОКУПНО ЛЕКАРСТВО Осн.1872

ГОДИШТЕ 145 ЈУЛ–АВГУСТ 2017. СВЕСКА 7–8

Часопис „Српски архив за целокупно лекарство" је индексиран у базама: Science Citation Index Expanded, Journal Citation Reports/Science Edition, Index Medicus (Medline, PubMed), Web of Science, Scopus, EBSCO, Directory of Open Access Journals, DOI Serbia.

Редакција
Технички уредник: Јасмина Живковић
Лектор за српски језик: Дивна Продановић
Лектор за енглески језик: Мирко Рајић
Корице и лого: Златко Т. Урошевић

Штампа: ЈП „Службени гласник", Београд

Тираж: 700 примерака

СРПСКИ АРХИВ
ЗА ЦЕЛОКУПНО ЛЕКАРСТВО Осн.1872

VOLUME 145 JULY–AUGUST 2017 ISSUE 7–8

The journal "Srpski arhiv za celokupno lekarstvo" (Serbian Archives of Medicine) is indexed in: Science Citation Index Expanded, Journal Citation Reports/Science Edition, Index Medicus (Medline, PubMed), Web of Science, Scopus, EBSCO, Directory of Open Access Journals, DOI Serbia.

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Technical editor: Jasmina Živković
Serbian language editor: Divna Prodanović
English language editor: Mirko Rajić
Cover & Logo: Zlatko T. Urošević

Printed by: JP "Službeni glasnik", Belgrade

Circulation: 700 copies

Srp Arh Celok Lek
ISSN 0370-8179
UDC 61(497.11)
COBISS.SR-ID 3378434
Serbian Archives of Medicine
Official Journal of the Serbian Medical Society
Published six times per year

FOUNDER, OWNER & PUBLISHER
Serbian Medical Society
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Serbian Medical Society
Džordža Vašingtona 19, 11000 Belgrade
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Bank accounts: 205-8041-21 and
355-1009094-22

Full-text articles are available at website: www.srpskiarhiv.rs

Calendar year subscription prices are as follows: 3,000 dinars for individuals, 6,000 dinars for institutions, and 100 Euros for readers outside Serbia. The price of a current issue is 600 dinars, and of a back issue 300 dinars.

The publishing of the Serbian Archives of Medicine during 2017 is supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

ISSN 0370-8179; ISSN Suppl 0354-2793

eISSN 2406-0895
Open Access
(CC BY-NC)

Printed in Serbia

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EDITORIAL • УВОДНИК

ORIGINAL ARTICLES • ОРИГИНАЛНИ РАДОВИ

DOI: https://doi.org/10.2298/SARH170710145R
UDC: 618.33

EDITORIAL / УВОДНИК

Intrauterine growth restriction: Causes and consequences

Nedeljko Radlović
Academy of Medical Sciences of the Serbian Medical Society, Belgrade, Serbia

The prenatal period of growth and development represents a very delicate phase of life exposed to numerous possible negative effects. One of them is intrauterine growth restriction (IUGR), also known as fetal growth restriction, i.e. birth with body weight below the 10th percentile in relation to the corresponding gestational age and sex [1]. The importance of adequate prenatal nutrition according to this optimal early development program was first pointed out by an English epidemiologist David Barker, less than three decades ago [2]. Namely, he noted the high association of IUGR with the occurrence of abdominal (visceral) obesity and metabolic syndrome in adulthood, i. e. insulin resistance, type II diabetes mellitus, and atherosclerosis and its consequences. Subsequently, only the confirmation of the "Barker hypothesis" in the narrow sense was followed by numerous evidence regarding the extremely high importance of early development programming and other aspects of human health [3–7]. The consequences of IUGR, as well as numerous other negative phenomena, are based on specific epigenetic mechanisms, such as DNA methylation, histone modification, and others, which by modifying gene expression result in a less functionally valuable and vulnerable phenotype [3, 6].

According to literature data, IUGR globally occurs with a prevalence of 3–10% [8]. The causes of this pathological phenomenon are numerous, such as placental abruption or abnormal insertion, maternal undernutrition, cigarette smoking, arterial hypertension, renal disease, anti-phospholipid syndrome, vitamin D deficiency and drug abuse, fetal chromosomal/structural anomalies or chronic intrauterine infection, and others [9–13].

In addition to the negative effects that occur in the adult age, IUGR complicates more frequent early postnatal problems such as asphyxia, hypothermia, hypoglycemia, polycythemia, and many more, when compared to their gestational age corresponding counterpart [5, 6, 8, 14]. Also, children who are born small for gestational age (SGA) have a predisposition to accumulation of fat mass, especially intra-abdominal [8]. Therefore, rapid weight gain during infancy of these children represents an additional risk factor for the occurrence of metabolic syndrome and its negative consequences in adult age [8].

The aforementioned facts point to the importance of IUGR as a worldwide health problem [8]. Although there are conditions where IUGR is an inevitable phenomenon, in many cases it can be prevented [6]. Although due to catch-up growth most of infants born SGA reach their peers in longitudinal growth at the age of two years, about 10% of them continue to fall below the third percentile of height into adulthood [8]. In order to eliminate additional risk factors for the subsequent occurrence of metabolic syndrome, it is important to point out that the nutrition of these infants must be focused on the prevention of obesity according to which they are strongly predisposed [6, 8].

Papers published in this issue of the Serbian Archives of Medicine deal with exclusive latest knowledge and make a significant contribution to clarifying these disorders, their early detection and treatment [15–19].

Received • Примљено: July 10, 2017
Accepted • Прихваћено: 12/7/2017
Online first: July 14, 2017

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DOI: https://doi.org/10.2298/SARH160205024M
UDC: 613.2.032.33-053.32

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effect of early introduction of minimal enteral feeding on growth and rate of achieving optimal nutritive intake in very low birth weight preterm infants

Vesna Marinković[1], Niveska Božinović-Prekajski[1], Milica Ranković-Janevski[1], Zorica Jelić[1], Vesna Hajdarpašić[1], Nedeljko Radlović[2,3,4]

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SUMMARY

Introduction/Objective Minimal enteral nutrition (MEN) has an important stimulative effect on morphological and functional development of gastrointestinal system in preterm infants.
The aim of this study was to assess effects of early introduced MEN on rate of achieving optimal enteral nutritive intake and on body weight, body length, and head circumference gain in very low birth weight (VLBW) premature infants.

Methods This prospective study included 45 VLBW newborns (1,010–1,450; 1,350 ± 305 g), in 30 newborns MEN was introduced within three days after birth, and in 15 newborns enteral intake was introduced after five days due to hemodynamic and metabolic instability. Assessment of effect of early MEN introduction on the rate of achieving optimal nutritive intake and gain in basic anthropometric parameters was based on comparison with a group of subjects who had a delayed MEN introduction.

Results Subjects in which MEN was introduced early on had better weight gain ($p < 0.05$), reached birth weight sooner ($p < 0.05$), and achieved optimal enteral intake much sooner ($p < 0.05$), compared to subjects with delayed MEN introduction. The difference in body length gain and head circumference gain was not significant.

Conclusion Early introduction of MEN has a significant positive effect on rate of body weight gain and on earlier achievement of optimal enteral intake in VLBW preterm infants.

Keywords: very low body weight infants; early minimal enteral nutrition; optimal nutritive intake

INTRODUCTION

With perinatal care improvements in the last few decades, incidence of neonatal morbidity and mortality has been significantly decreased. Very important role goes to adequate nutrition of these very vulnerable children, and its positive effects reflect not only on survival rate and optimal growth and development, but on adulthood as well [1].

The development of gastrointestinal system starts early in the intrauterine period and continues postnatally. Although highly immature, morphologically and functionally, gastrointestinal system partially meets, initially very poorly, basic nutritive needs of premature infant. In a very complex process of progressive postnatal functional maturation of the gastrointestinal system, early introduction of enteral feeding has a key role as a physiological stimulus [1, 2].

During the 1980s, new tendencies arose advocating early initiation of enteral nutrition, which led to the abandonment of long-standing practice of delayed enteral feeding of premature infants in intensive care units [1, 2]. Minimal enteral nutrition (MEN) implies early intake of primarily mother's milk in small amounts (up to 25 mL/kg/day) in premature infants [2]. This kind of nutrition doesn't primarily provide optimal nutritive balance in premature infants. Its basic role is contained in trophic influence on the immature gastrointestinal system, i.e. on the development of process of food digestion and absorption, coordination of motility, gastrointestinal hormone activity, and on preservation of intestinal barrier integrity. In that manner better feeding tolerance is achieved, as well as faster postnatal growth and development, lower incidence of sepsis and necrotizing enterocolitis, and shorter hospitalization [2]. Nowadays, this way of nutrition is generally accepted in most neonatal intensive care units as an integral part of treatment of premature infants [3].

The effects of early introduction of MEN are assessed in regard to body weight (BW), body length, head circumference, and rate of achievement of optimal nutritive intake in very low birthweight premature infants.

Received • Примљено: Februry 5, 2016
Accepted • Прихваћено: March 7, 2016
Online first: February 21, 2017

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METHODS

This prospective study included 45 very low birth weight (VLBW) premature infants hospitalized at the Institute for Neonatology, Belgrade, Serbia, since June 2012 until September 2013. The subjects were divided into two groups according to the time of MEN introduction: group A, n = 30, in which after establishing hemodynamic stability, normal blood pressure, blood pH above 7.3 and FiO_2 under 40%, MEN was introduced within three days after birth; and group B, n = 15, in which MEN was introduced 5–10 days (average 6.07) after birth due to hemodynamic and metabolic instability and/or meteorism ($t = 0.02$, $p < 0.05$). Basic characteristics of both groups of subjects are presented in Table 1. Distribution of subjects according to sex was identical between groups. Group A had 14 male subjects (46.7%) and 16 female subjects (53.3%), and group B had seven male (46.7%) and eight (53.3%) female subjects.

The basic way of meeting nutritive needs in both group of subjects during the first days of life was via parenteral nutrition, while enteral feeding via nasogastric tube was introduced according to the above mentioned criteria. Enteral nutrition in all newborns was started with human milk, be it donor milk or mother's milk. Daily intake volume in the first five days was 25 mL/kg/day, and was increased gradually according to feeding tolerance. When enteral volume intake of 80 mL/kg/day was achieved, fortified human milk was introduced (FM 85 Nestlè, Vevey, Switzerland) in a dose of 5 g / 100 mL, and/or specialized milk formula for premature infants (Mil PRE, Impamil d.o.o., Belgrade, Serbia).

The following parameters were followed in all subjects: daily gain in body weight, day of achieving birth weight, weekly increase in body length and head circumference, and day of achieving optimal enteral intake.

All data obtained during research was analyzed with SPSS 10.0 for Windows software package. Both parametric and non-parametric statistical tests were used to analyze the data. Comparison of the two groups was done using Student's t-test and Mann–Whitney U-test, depending on the data homogeneity. Comparison of data between more than two groups was done with Kruskal–Wallis test. Statistical significance was set at $p < 0.05$.

RESULTS

Subjects with early introduced MEN (group A) compared to subjects with delayed introduction of MEN (group B) had a significantly better body weight gain (10.88 ± 3.25 g vs. 7.73 ± 1.85 g daily; $t = 0.017$, $p < 0.05$), and achieved birth weight sooner (16.38 ± 3.36 days vs. 21 ± 7.16 days; $t = 0.017$, $p < 0.05$) (Figure 1). Also, group A subjects achieved optimal enteral intake significantly sooner compared to group B (25.7 ± 7.2 days vs. 28.33 ± 7.35 days; $t = 0.021$, $p < 0.05$) (Figure 2).

The difference in weekly gain in body length during the observed period between subjects in group A (0.45–0.58; 0.51 ± 0.35 cm) and group B (0.45–0.53; 0.49 ± 0.33 cm) was not significant ($t = 0.025$, $p > 0.05$).

The difference in weekly head circumference gain between group A (0.46–0.67; 0.49 ± 0.21 cm) and group B (0.44–0.53; 0.5 ± 0.21 cm) during the observed period was also not significant ($t = 0.022$, $p > 0.05$).

DISCUSSION

Because of its stimulating effect on morphological and functional development of the gastrointestinal system,

Table 1. Basic characteristics of subjects at birth (n = 45)

Characteristics	Group A (n = 30)		Group B (n = 15)	
	Range	($\bar{x}$ ± SD)	Range	($\bar{x}$ ± SD)
Birth weight (g)	1,000–1,500	1,274.0 ± 144.6	1,000–1,500	1,250 ± 152.2
Gestational age (weeks)	26–32.5	29.5 ± 1.6	27–31.5	29.0 ± 1.2
Apgar score 1′	1–8	4.9 ± 1.9	2–8	5.53 ± 1.9
Apgar score 5′	2–8	5.9 ± 1.5	4–8	6.33 ± 1.5

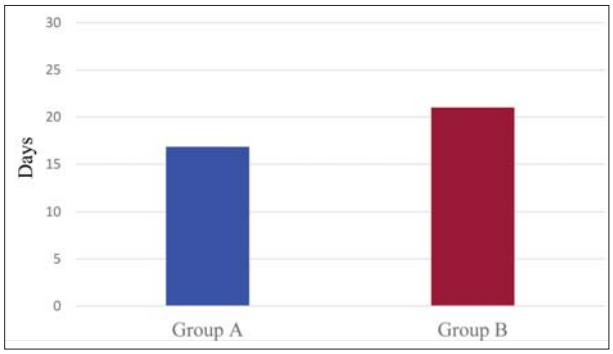

Figure 1. Achievement of birth weight (day)

A:B; $p < 0.05$

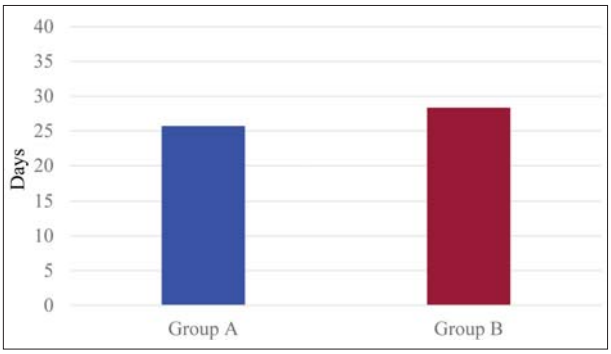

Figure 2. Achievement of optimal nutrition intake (day)

A:B; $p < 0.05$

MEN introduced during the first 24–72 hours after birth is also known in literature as „trophic feeding" [4]. Some authors consider early introduction of MEN to be within four days after birth [5].

In our subjects, MEN was introduced within the first 72 hours as advocated by most authors [4]. Amount of enteral intake was also a problem in everyday work, because it varied greatly between studies. Therefore, MEN was defined as small volume enteral intake, up to 25 mL/kg/day, or less than 20 kcal/kg/day [6]. MEN was introduced in our subjects in accordance with the aforementioned recommendation.

Precondition for the initiation of MEN is clinical state of a patient, his/her metabolic and hemodynamic stability. Precaution should be taken in case of severe perinatal asphyxia, sepsis, severe hemodynamic instability, absence of end-diastolic flow, indomethacin therapy and hemodynamically significant persistent ductus arteriosus, because of possible necrotizing enterocolitis development [7]. Before enteral feeding was introduced in our subjects, their mean arterial pressure was within reference range for body weight and gestational age, there was no meteorism, and pH value exceeded 7.3.

It is fully understood nowadays that MEN should be initiated with mother's milk, using colostrum whenever possible [3]. Otherwise, when mother's milk is not available, human donor milk from a milk bank is an optimal choice [8]. Current tendencies show the need for establishing human milk banks which should be the foundation of nutritive support of premature infants and can contribute greatly to lactation preservation [9]. It is general attitude that mother's milk, with appropriate supplementation, represents the foundation of nutrition of preterm infants. Unfortunately, in most cases, production of human milk is inadequate or lactation is not established at all, in which case nutrition with donor milk is appropriate choice [9, 10]. In all our subjects, MEN was conducted with human donor milk, in which colostrum was used in five subjects.

The duration of MEN and further increase in volume intake are also not precisely defined. There is a need for a unique protocol considering increase in volume intake which would have primarily practical role in everyday neonatologist's work [11]. In our subjects, MEN was conducted over a period of days, with a volume of up to 25 mL/kg/day. After this time, an increase in volume intake was 15–20 mL/kg/day, adjusted to individual feeding tolerance.

Measurements of body weight, body length, and head circumference represent basic anthropometric indicators of growth in the neonatal period. Skinfold thickness and subscapular test are far less significant in neonatal clinical practice, considering very small changes in neonates [12]. Proper technique of measurement and adequately trained personnel need to perform measurements in intervals prescribed by the protocol or research methodology. During this study, body weight was measured on digital scales incorporated in incubators or on classic mechanical scales (accuracy range ± 5 g). The proper way of measuring body length is by using a stadiometer, but depending on the clinical state of a patient, various forms of adapted flexible plastic-coated tape measure are used. The use of tape measure made from impregnated unstretchable cloth is the most optimal way of measuring head circumference [13].

The time of birth weight achievement is also an indirect indicator of nutritive support, and it is three weeks in VLBW preterm infants, but according to clinical condition it can be even longer [14]. Our subjects in whom MEN was introduced early on reach birth weight on day 17, which is significantly shorter compared to day 21 in group with delayed enteral nutrition ($p < 0.05$).

Body weight gain during intrauterine growth is about 15–20 g/kg/day, while postnatal growth of 10–20 g/kg/day is considered to be appropriate [15]. Average body weight gain in the group with early introduced MEN was 10.88 g/kg/day, which is significantly more compared to 7.73 g/kg/day in the group with delayed enteral intake ($p < 0.05$).

Increase in body length and occipitofrontal head circumference of 0.9 cm per week is ideal, and represents a goal of adequate nutritive support, although this value is far lower and harder to reach in clinical practice. Monitoring of early postnatal growth through series of body length measurements in preterm infants shows a value of 0.5–0.9 cm per week, and occipitofrontal head circumference of 0.5–1.1 cm per week [16, 17, 18]. Average weekly gain in body length in our subjects was 0.45–0.58 cm, and in head circumference 0.46–0.67 cm. The conducted study wasn't coherent considering the question of effect of minimal enteral nutrition on short-term growth, while analysis of long-term growth and its developmental effects was not analyzed [19]. There is a need for new randomised studies that will include extremely low birth weight infants in this research, as well as infants with intrauterine growth restriction [20, 21].

CONCLUSION

Minimal enteral nutrition with human milk as an addition to parenteral nutrition represent a very important practical approach in treatment of VLBW premature infants, naturally stimulating the development of gastrointestinal functions. Minimal enteral nutrition introduction within 72 hours compared to five or more days after birth significantly contributes to the rate of body weight gain and to earlier achievement of optimal nutritive intake, so it should be practiced whenever possible.

NOTE

This paper is a part of a master's thesis titled "Analysis of the effect of minimal enteral nutrition on the growth of very low birthweight premature infants," defended on February 18, 2014 at the School of Medicine, University of Belgrade.

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Утицај ране минималне ентералне исхране на раст и брзину постизања оптималног нутритивног уноса превремено рођене деце веома мале телесне масе

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Увод/Циљ Минимална ентерална исхрана (МЕИ) има битан стимулативни ефекат на морфолошки и функционални развој гастроинтестиналног система код превремено рођеног детета.

Циљ рада била је процена ефеката ране МЕИ на брзине постизања оптималног ентералног нутритивног уноса и раст телесне масе, телесне дужине и обима главе код превремено рођене деце веома мале телесне масе (ВММТМ).

Методе рада Проспективном студијом је обухваћено 45 новорођенчади ВММТМ (1.010–1.450; 1.350 ± 305 *g*), 30 код којих је МЕИ започет унутар три дана по рођењу и 15 код којих је због хемодинамске и метаболичке нестабилности ентерални унос започет након пет дана. Процена ефекта ране МЕИ на брзину постизања оптималног нутритивног уноса и раст основних антропометријских параметара анализирана је на поређењу са групом испитаника код којих је ентерални унос започет касније.

Резултати Испитаници са рано започетом МЕИ у односу на оне код којих је ентерална исхрана одложена су боље напредовали у телесној маси ($p < 0,05$), брже достизали порођајну телесну тежину ($p < 0,05$) и знатно раније успостављали оптимални ентерални унос ($p < 0,05$), док разлика у расту телесне дужине и обима главе између ове две групе испитаника није била значајна.

Закључак Рана МЕИ има знатан позитиван ефекат на брзину пораста телесне тежине и раније успостављање оптималног ентералног уноса код превремено рођене деце ВММТМ.

Кључне речи: новорођенчад веома мале телесне масе; рана минимална ентерална исхрана; оптимални нутритивни унос

DOI: https://doi.org/10.2298/SARH161124090T
UDC: 616.61-074-053.32

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Serum chloride and sodium concentration as a predictor of acute kidney injury in premature newborns

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SUMMARY

Introduction/Objective Hyperchloremia is often registered in adults' studies after administration with 0.9% sodium chloride, which contributes to the development of acute kidney injury (AKI) as it leads to vasoconstriction of renal blood vessels.

The aim of this study was to determine the correlation of sodium and chloride imbalance with the development of AKI, with consideration of other risk factors for this disorder.

Methods This retrospective study included 146 randomly selected preterm infants hospitalized at the Neonatal Intensive Care Unit from 2008 to 2015.

Results Among the patients registered for the study, 23.97% developed AKI, and they were of a significantly lower gestational age (26.3 ± 2.8 weeks vs. 31.7 ± 2.90 weeks, $p < 0.05$); birth weight (971.31 ± 412.1 g vs. 1,753.3 ± 750.3 g, $p < 0.05$); Apgar score in the first (3.2 ± 1.7 vs. 5.7 ± 2.4, $p < 0.05$) and fifth minute (5.3 ± 1.7 vs. 7.1 ± 1.8, $p < 0.05$) of life compared to those without AKI. The neonates with AKI had significantly higher maximum chloremia (Cl_{max}: 114.1 ± 8.4 vs. 111.7 ± 4.6, $p = 0.029$) and maximum natremia (Na_{max}: 147.9 ± 8.8 vs. 142.9 ± 4, $p < 0.05$). Each of these parameters is (independently) a statistically significant risk factor for the development of AKI, and gestational age is the strongest (OR = 1 / 0.643 = 1.55; 95% CI 1.24–1.94). Mortality in neonates with AKI was higher than in neonates without AKI (19.4% vs. 92.7%, $p < 0.05$).

Conclusion Hyperchloremia and hypernatremia are more common in the premature newborns with AKI compared to the premature newborns without AKI. Higher maximum sodium and chloride values are independent risk factors for AKI.

Keywords: acute kidney injury; hyperchloremia; hypernatremia; premature newborns

INTRODUCTION

Acute kidney injury (AKI) is a rapid decline of renal function, represented by reduction of glomerular filtration rate (GFR) with the accumulation of nitrogen substances and dysregulation of extracellular fluid, electrolyte, and acid-base balance [1]. Newborns have a low GFR, which limits renal adaptation to different stress factors, making them vulnerable to the development of AKI. The vulnerability is more pronounced in preterm infants or those with low birth weight or with intrauterine growth restriction [2]. The frequency of AKI in neonates in a neonatal intensive care unit (NICU) ranges from 8% to 24%, and a third of them are preterm. Mortality due to AKI is high, and AKI is most often nonoliguric (in about 60%) [1].

Attempts have been made recently to clarify the diagnostic criteria for AKI in neonatal population, especially in premature neonates, where early detection can lead to improved outcomes. The current RIFLE criteria for the diagnosis of AKI by ADQI (Acute Dialysis Quality Initiative) are based on an increase in serum creatinine levels with or without changes in the urine output. Their modification for the pediatric population established lower levels of creatinine for diagnosis of AKI in children, but it still lacks clear levels of creatinine in neonatal age, especially in preterm neonates [1]. In the first three days of life, the value of the infant's creatinine level correlates with that of the mother, and its value declines quickly during the first days of life. According to AKIN (Acute Kidney Injury Network) criteria for AKI diagnosing, the absolute value of creatinine is introduced, and AKI is classified into three stages of severity depending on the level of creatinine. Considering additional specificity of the neonatal population (especially preterm) and the immaturity of tubular cells, a higher percentage of body water and higher normal urinary output were proposed by some studies [1, 3, 4]. Presently, there is no unique and completely reliable definition for AKI in neonates.

Significant association between lower birth weight and gestation age, perinatal asphyxia, respiratory distress syndrome, phototherapy, patent ductus arteriosus, lower Apgar score levels, use of drugs in mother and newborn (NSAIDs, antibiotics), sepsis with the development of AKI has been confirmed in several

Received • Примљено: November 24, 2016
Accepted • Прихваћено: March 14, 2016
Online first: March 28, 2017

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studies [1, 3]. The most common form of AKI in prematurely born infants is prerenal (85%), associated with ischemia, hypoxia, and hypovolemia [1, 2]. Hypovolemia develops due to dehydration, fluid loss in the "third" space due to sepsis, perinatal hemorrhages, gastrointestinal losses, hypoalbuminemia or hypovolemia maintained due to cardiomyopathy and other reasons. If not treated in a timely manner, hypovolemia and ischemia progress and lead to damage of nephrons [1, 2, 3].

Standard therapy of AKI includes maintaining the volume of circulating fluid through the infusion solution, but with caution against excessive compensation volume and electrolyte abnormalities, limiting intake of nephrotoxic drugs and other supportive therapy [1, 3]. Infusion therapy takes crucial place for the maintenance of renal perfusion and GFR, prevention and treatment of AKI. The standard infusion used in neonatology is 0.9% sodium chloride and 5% glucose.

There has been much discussion in the past few decades about the negative effects of hyperchloremia registered with the infusion of 0.9% sodium chloride, including the development of hyperchloremic acidosis and negative effect on renal blood vessels especially, leading to their vasoconstriction [5, 6]. This vasoconstriction further reduces GFR.

The aim of our study was to determine the value of chloride and sodium in the serum of premature infants, as well as association of electrolyte imbalances with the development of AKI, at the same time taking into consideration other risk factors for this disorder.

The objective of the present study is to determine the correlation of sodium and chloride imbalance with the development of AKI, with consideration of the other risk factors for this disorder.

METHODS

The retrospective study included information from medical records of 146 randomly chosen premature newborns (born before the 37th week of gestation) hospitalized at the NICU of the Institute for Child and Youth Health Care of Vojvodina (ICYHCV) in the period from January 1, 2008 to December 31, 2015.

Access to medical records was approved by the Ethics Committee of ICYHCV.

The study does not include premature infants who had congenital malformation of the urinary tract or other congenital disorders that could directly or indirectly affect the serum sodium and chloride (congenital adrenal hyperplasia, Bartter syndrome, hydrocephalus, cardiomyopathy), infants who died in the first 72 hours of treatment, nor infants with incomplete medical records.

All respondents were divided into two groups: those with AKI (n = 35) and infants without AKI (n = 111). The following information was gathered from medical records: gestational age, birth weight, Apgar score at the first and the fifth minute, sex, the requirements for mechanical ventilation and for non-invasive respiratory support, the length of stay on NICU and entire hospital stay, outcome, and the serum chloride and sodium levels. The length of stay in the NICU is expressed as the sum for patients who stayed at the NICU more than once during their hospitalization, and the length of hospital stay was calculated only for surviving patients.

For the assessment of possible correlation between serum chloride and sodium with AKI in the group of infants who developed AKI, we calculated chloride and sodium values before the development of AKI. We analyzed initial value of chloride on admission in NICU, marked Cl_0, the maximum recorded value of chloride (Cl_{max}), minimum (Cl_{min}), and average (Cl_{mean}) value of chloride. The sodium levels were analyzed in the same way: initial (Na_0), maximum (Na_{max}), minimum (Na_{min}), and average (Na_{mean}) value.

AKI was diagnosed according to the modified AKIN criteria. Increase in serum creatinine of ≥ 26.4 mmol/l (≥ 0.3 mg/dl) compared to the baseline value of the third day of life or if the basal value was not determined, an increase of the serum creatinine of ≥ 26.4 mmol/l (≥ 0.3 mg/dl) within 48 hours was defined as AKI.

The reference range of the chloride is 98–108 mmol/l and for sodium 135–145 mmol/l.

The parameters of descriptive statistics are presented through the mean value ± standard deviation or percentage and median and interquartile range (IQR). Statistical comparisons were made by Student's t-test, χ^2 test or Mann–Whitney U-test. Univariate and multivariate logistic regression models were conducted for evaluating the prediction for the development of AKI for all variables. For statistically significant difference, values with $p < 0.05$ were taken.

RESULTS

Out of all of the preterm infants, (n = 146) 35 (23.97%) had AKI. AKI was diagnosed on average after 5.5 ± 4.2 days of life. Compared with premature infants without AKI, neonates with AKI were of a significantly lower gestational age (26.3 ± 2.8 weeks vs. 31.7 ± 2.9 weeks, $p < 0.05$), as well as of a lower birth weight (971.31 ± 412.1 grams vs. 1,753.3 ± 750.3 grams, $p < 0.05$), had lower values of Apgar score in the first (3.2 ± 1.7 vs. 5.7 ± 2.4, $p < 0.05$) and fifth minute (5.3 ± 1.7 vs. 7.1 ± 1.8, $p < 0.05$) of life. Mechanical ventilation was more frequently applied in neonates with AKI compared to those without AKI (94.3% vs. 67.8%, $p < 0.05$), and the neonates without AKI were more often on a non-invasive respiratory support (67.8% vs. 34.3%, $p < 0.05$). Stay of the patients with AKI in the NICU was significantly longer [17 (14, 29.5) days vs. 8 (4, 13) days, $p = 0.006$] and entire hospital stay [52 (42, 68) days vs. 36 (20.5, 55) days, $p = 0.047$] by analyzing only survivors in both groups. Survival was significantly higher in the neonates without AKI compared to the neonates with AKI (92.7% vs. 19.4%; $p < 0.05$) (Table 1).

The neonates with AKI had significantly greater maximum values of chloride (Cl_{max} = 114.1 ± 8.4 vs. 111.7 ± 4.6, $p = 0.029$) and maximum values of sodium (Na_{max} = 147.9 ± 8.8 vs. 142.9 ± 4, $p < 0.05$) compared to the neonates

Table 1. Descriptive statistics and differences between groups of infants with and without AKI

PARAMETERS		All (n = 146)	AKI (n = 35)	Without AKI (n = 111)	p
GA (weeks)		30.6 ± 3.5	27 ± 2.8	31.7 ± 2.91	< 0.05
BW (gr)		1,565.9 ± 761	971.31 ± 412.1	1,753.3 ± 750.3	< 0.05
AS 1st min.		5.2 ± 2.5	3.2 ± 1.7	5.7 ± 2.4	< 0.05
AS 5th min.		6.8 ± 1.8	5.3 ± 1.7	7.1 ± 1.8	< 0.05
Gender (male, %)		88 (60.3)	20 (55.6)	68 (60.7)	0.188
Respiratory support	MV (n, %)	109 (74.6)	33 (94.3)	76 (67.8)	< 0.05
	Noninvasive (n, %)	96 (65.7)	12 (34.3)	76 (67.8)	< 0.05
Survivor (n, %)		111 (76)	7 (19.4)	103 (92.7)	< 0.05
Length of stay	NICU (days; median, IQR)	8.5 (4.25, 15)	17 (14, 29.5)	8 (4, 13)	0.006
	Entire hospital stay (days; median, IQR)	37 (21.25, 55.75)	52 (42, 68)	36 (20.5, 55)	0.047

GA – gestational age; BW – birth weight; AS 1 and 5 – Apgar score in the first and fifth minute; MV – mechanical ventilation; IQR – interquartile range; NICU – neonatal intensive care unit

without AKI. Table 1 shows the initial, minimum, and mean values of chloride and sodium levels in the neonates with and without AKI.

A univariate logistic regression model was developed for each variable for the prediction of AKI. It was found that Cl_{max}, Na_{max}, gestational age, Apgar score in the first and fifth minute, birth weight, and the use of mechanical ventilation were statistically significant independent risk factors for the development of AKI (Table 1). For each increased unit of Cl_{max}, the risk of AKI is 1.071 higher (OR 1.071, 95% CI: 1.005–1.141, p = 0.036), and for each increased unit of Na_{max} 1.14 (OR 1.14, 95% CI: 1.068–1225, p < 0.05).

By switching off the parameters, which mutually demonstrated a strong correlation (gestational age in relation to birth weight r = 0.860, p = 0.000; Apgar score in the first and fifth minute, r = 0.860, p = 0.000), and taking only one of them, such as gestational age and value of the Apgar score in the first minute, a multivariate logistic analysis showed that the strongest predictor of development AKI is gestational age. Decreasing the gestational age for one week increased the risk of AKI in premature infants up to 1.55 times (OR = 1 / 0.643 = 1.55, 95% CI: 1.24–1.94) (Table 1).

DISCUSSION

AKI is associated with high mortality and occurs frequently in the NICU. The most important risk factors for the development of AKI, beside those which lead to poor renal perfusion and hypoxia or nephrotoxicity, were prematurity and low birth weight [1, 3].

In a retrospective study by Stojanović et al. [7], which analyzed 150 premature infants treated in the NICU, 26% of analyzed infants (39/150) developed AKI. In the group of neonates with AKI significantly lower gestational age was recorded (27.3 weeks vs. 31.3 weeks, p < 0.001) and lower birth weight (1,034 g vs. 1,620 g, p < 0.001), lower Apgar score at birth in the first and in the fifth minute and significantly higher comorbidity (sepsis, high stage of intracranial hemorrhage, necrotizing enterocolitis, patent ductus arteriosus) in comparison with the preterm neonates without AKI. The final outcome was significantly worse in those with AKI, as the number of deaths was 69.2% vs. 13.5% (p < 0.001) in the neonates without AKI.

Koralkar et al. [8] analyzed newborns with low birth weight (≤ 1,500 g), treated at an NICU, in a prospective study. It was found that 18% of the neonates developed AKI and that they had lower values of Apgar score in the fifth minute and were of significantly lower birth weight (702 g vs. 1,039 g, p < 0.001), and lower gestational age (25 vs. 28, p < 0.001). As the gestational age was lower, they had a higher stage of AKI by AKIN criteria. Mortality in neonates with AKI was significantly higher than in those without AKI (42% vs. 5%, p < 0.001).

In our group of neonates, AKI was registered in 23.97%, but only 19.4% of the patients survived. They were of significantly lower gestational age and lower birth weight, and had lower values of Apgar score in the first and fifth minute of life and needed more use of mechanical ventilation. All of these parameters proved to be independent contributing factors for the development of AKI.

The role of hyperchloremia as a risk factor for AKI in neonates has not been studied. In adult intensive care units, significant volume replacement after major surgery or trauma has been shown to lead to hyperchloremia due to higher volume replacement with solutions rich in chloride, and this hyperchloremia has not been recorded in the volume replacement of the balanced solution [5]. Through various studies, both experimental and clinical, evidence suggests that hyperchloremia reduced renal perfusion and GFR.

Shaw et al. [6] showed that the use of 0.9% sodium chloride in 30,994 patients during open abdominal surgery carries a higher risk of complications than in the group of 926 patients who received a balanced crystalloid solution with a reduced content of chloride on the day of the operation. In patients who received only the infusion of 0.9% sodium chloride on the day of surgery, significantly more infections, electrolyte imbalance, development of acidosis, and the development of acute kidney failure were recorded and they had more need for renal replacement therapy, and more need for higher volume replacement and blood

transfusions. The mortality rate among patients who received 0.9% sodium chloride was 5.6%, compared with 2.9% in the group of patients who received balanced crystalloid. An unfavorable result in terms of higher percentage of hyperkalemia and acidosis was noted in the application of 0.9% sodium chloride in patients who underwent kidney transplantation, compared with those who received Ringer's lactate, until there was no significant difference in renal function [9]. Zhang et al. [10] in the study of 1,221 critically ill adult patients showed that those who had developed AKI (29.2%) during their stay in the intensive care unit had significantly higher values of chloremia compared with the patients without AKI, that there is a correlation between the Cl_{max} and Cl_{mean} values with the development of AKI, and that Cl_{max} was significantly higher in severe stages of AKI.

In our study group, we found that premature infants who developed AKI had significantly higher values of Cl_{max} and Na_{max} compared to the to the control group and that these parameters are independent risk factors for the development of AKI.

Hypernatremia indicates a state of dehydration caused by physiological predisposition of premature neonates, as well as the presence of pathology. Immature neonates with lower birth weight lose more water through perspiration; another significant way of water loss among neonatal population is infection, when the fluid exceeds into "third space." Stojanović et al. [7] performed a study in which daily fluid intake in prematurely born neonates was monitored. For those who developed AKI, it was noted that they had higher levels of sodium in the third and fourth day of life. This major difference of sodium levels indicates a higher degree of dehydration in the premature neonates with AKI.

In the present study, in both groups we applied solution which contained 0.9% sodium chloride and 5% glucose, to maintain fluid balance. Recommendations for infusion therapy in neonatology suggest a better outcome with a restrictive fluid replacement because GFR and concentration ability of kidneys in premature infants in the first days of life are reduced, and urine output is limited to 0.5 ml/kg/h in the first 12–24 hours [7]. Our assumption is that hyperchloremia in prematurely born infants with AKI occurred primarily because of parenteral rehydration with infusion solution rich in chloride.

An experimental study on the effect hyperchloremia has on kidneys on anesthetized dogs has was made a few decades ago. In the study, Wilcox [11] described renal vasoconstriction and GFR decline in the denervated kidneys dependent on the level of hyperchloremia. He applied captopril to block angiotensin II in an attempt to explain the mechanism of vasoconstriction. However, other studies have shown that angiotensin II vasoconstrictor is not important during infusion rich in sodium chloride because the application does not block renal vasoconstriction. The infusion of sodium chloride increases interstitial fluid more in comparison to balanced crystalloid solution. The result of this is that at relatively stretchable tissue, for example, in encapsulated organs, little accumulation fluid results with elevated intracapsular pressure, which then reduces tissue perfusion and slows down capillary flow [5]. This mechanism can serve as one of the explanations for the reduced diuresis after the infusion of sodium chloride. It was an experiment that demonstrated improvement of renal function and an increase in urine output during massive preoperative compensation volume (crystalloid and blood transfusion) in hypovolemic monkeys who underwent kidney decapsulation [12]. Namely, kidney decapsulation was prevented of secondary renal ischemia due to compartmental swelling. Hypovolemia, pain, and various stressful factors activate the sympathetic nervous system and the renin-angiotensin-aldosterone system (RAAS). Consequently, the activated and enhanced secretion of antidiuretic hormone leads to the retention of water and sodium. Even in conditions without damage or kidney lesions, the connection between fluid intake and natriuresis is weak and the application of infusion is more likely to lead to water and sodium retention rather than to diuresis [5]. Studies of the general population have been conducted in order to monitor physiological events in the intake of sodium chloride. In a double-blind controlled study that involved healthy volunteers, by using magnetic resonance imaging, it was observed that there was a decrease of cortical perfusion and reducing flow rates in the renal artery after 2 l infusion of 0.9% sodium chloride compared with 2 l infusion Plasma-Lyte 148 solution [13]. Drummer et al. [14] have shown that in healthy volunteers, introduction of 2 l of 0.9% sodium chloride solution for 25 minutes was followed by increased excretion of urine and electrolytes for the next two days, with the biggest increase 3–22 hours after the infusion. Two days after the infusion they noted the suppression of RAAS, which corresponds to the influx of sodium, whereas atrial natriuretic peptide and urodilatin were raised 22 hours after the infusion, suggesting that they may represent a slower adaptive mechanism of the prolonged infusion with sodium chloride.

In their work, which involved 3,603 healthy 25–75-year-old individuals, Nakajima et al. [15] pointed out that there is a link between elevated levels of sodium and high blood pressure, but also that higher serum sodium is associated with reduced GFR independently of high blood pressure. Changes in serum chloride significantly contribute to high blood pressure through high concentration of sodium, but not to the correlation between the high concentrations of sodium and GFR decrease. Within one hour after sodium chloride infusion, RAAS is suppressed, but not after the infusion of sodium bicarbonate. In the experimental model of sodium sensitive hypertonic rats, hypertension was shown to be under the influence of sodium chloride, but not of sodium bicarbonate [16].

It is considered that mechanism by which hyperchloremia leads to vasoconstriction includes signaling of macula densa cells. Due to increased concentrations of sodium chloride in the renal tubules, more sodium and chloride enter macula densa cell through $NaCl_2K$ cotransporter, then chloride exits through the chloride channels on the basolateral side of macule densa cell causing its depolarization, which leads to the release of adenosine triphos-

phate (ATP) and vasoconstriction of afferent and efferent arterioles. It is assumed that ATP has a direct effect, but also adenosine, a decomposition product of ATP, acting on A1 receptors leads to the vasoconstriction of afferent and efferent arterioles [5, 17]. There are also other possible mechanisms for negative effects of hyperchloremia [13].

Considering the specificities of the neonatal population, premature infants may be especially susceptible to the effects hyperchloremia. According to previous studies, more ATP was found in erythrocytes of infants than in those of adults, and more ATP in prematurely born infants than in term infants [18]. Hypothetically, if cells of macula densa in premature infants have more ATP, their depolarization probably involves more ATP release, and thus a stronger vasoconstriction of afferent and efferent arterioles. There are no clinical studies on the effects of hyperchloremia in neonates. Completion of the research in this area is certainly necessary to extend our knowledge.

CONCLUSION

In our group of neonates, premature infants with AKI had poor outcome. Lower gestational age, lower Apgar score in the first and fifth minute, lower birth weight, and the use of mechanical ventilation are significant independent risk factors for the development of AKI. A significant contribution to the development of AKI was found for the maximum value of chloride and sodium, which were significantly higher in the group of preterm infants who developed AKI. A larger study is needed to determine which fluid is most appropriate for use in premature newborns.

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Концентрација хлора и натријума у серуму као предиктор развоја акутног оштећења бубрега код превремено рођене новорођенчади

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САЖЕТАК

Увод/Циљ У студијама на одраслима утврђено је да након надокнаде течности физиолошким раствором долази до хиперхлоремије, која доводи до вазоконстрикције бубрежних крвних судова и развоја акутног оштећења бубрега (АОБ). Циљ рада је био да се утврди повезаност дисбаланса хлора и натријума у серуму са развојем АОБ-а, као и факторе ризика који предиспонирају АОБ.

Методе У ретроспективну студију је укључено 146 насумично изабране превремено рођене деце лечене на Одељењу неонаталне интензивне неге од 2008. до 2015. године.

Резултати Од укупног броја новорођенчади (n = 146), 23,97% је развило АОБ. Новорођенчад са АОБ-ом су имала значајно нижу гестацијску старост (26.3 ± 2.8 одн. 31,7 ± 2.90 недеља, $p < 0.05$), порођајну тежину (971,31 ± 412,1 *gr* тј. 1753,3 ± 750,3 *gr*, $p < 0,05$), Апгар скор у првом (3,2 ± 1,7 тј. 5,7 ± 2.4, $p < 0,05$) и петом минуту живота (5,3 ± 1,7 тј. 7,1 ± 1.8, $p < 0,05$) у поређењу са децом без АОБ-а. Новорођенчад са АОБ-ом су имала значајно веће максималне вредности хлора у серуму – 114,1 ± 8,4 тј. 111,7 ± 4.6, $p = 0,029$, као и максималне вредности натријума у серуму – 147,9 ± 8,8 према 142,9 ± 4, $p < 0,05$. Сваки од ових параметара је независни статистички значајан фактор ризика за развој АОБ-а, а гестацијска старост је најзначајнији (*OR* = 1/0,643 = 1,55, 95% *CI* 1,24 до 1,94). Морталитет је био значајно већи код новорођенчади са АОБ-ом у односу на децу без АОБ-а (19,4% тј. 92,7%, $p < 0,05$).

Закључак Хиперхлоремија и хипернатремија су чешће код превремено рођене новорођенчади са АОБ-ом у поређењу са децом без АОБ-а. Веће максималне вредности натријума и хлора у серуму су независни фактори ризика за развој АОБ-а.

Кључне речи: акутно оштећење бубрега; хиперхлоремија; хипернатремија; превремено рођено новорођенче

DOI: https://doi.org/10.2298/SARH160818108X
UDC: 618.39(510)"2012"; 616-053.32(510)"2012"

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Causes and short-term outcomes of preterm infants

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SUMMARY

Introduction/Objective Preterm birth (PB) is the most important reason of neonatal mortality, and the second most common direct cause of death for children under the age of five years.
The aim of this study was to analyze the clinical features and outcomes of preterm infants.

Methods The clinical data of 307 preterm infants delivered in the Qingdao University hospital from January 1, 2012 to December 31, 2012 were retrospectively analyzed.

Results The incidence of PB was 6.52%. There were 143 cases of preterm prelabour rupture of membranes (PPROM) (46.58%), 66 cases of spontaneous PB (21.5%), and 98 cases of therapeutic PB (31.92%). Deliveries with gestational weeks (GW) < 32 were mainly vaginal (60.72%), but deliveries with GW ≥ 32 exhibited higher C-section rate (60.99%) than the vaginal delivery rate ($p < 0.05$). The birth weight was 2,340.46 ± 606.26 g, and the Z-score at birth was -0.15 ± 1.08. The Z-score in the group with GW within 28 to 31^{+6} weeks was less than that in the group with GW within 32 to 33^{+6} and with GW ≥ 34 ($p < 0.05$). The average hospital stay of preterm infants was 15.17 ± 12.35 days, and the most common complication in these preterm infants was respiratory distress syndrome with 13.92%.

Conclusion PB could cause a variety of serious complications in infants. The main causes of PB, such ac PPROM, should be actively prevented and treated; meanwhile, preterm infants should also be actively treated so as to improve their outcomes.

Keywords: infant; pregnancy outcomes; preterm birth; Z score

INTRODUCTION

Preterm birth (PB) refers to the delivery with less than 37 gestational weeks, and it is estimated that there are more than 41,000 cases of preterm delivery daily in the world [1]. PB is the most important reason of neonatal mortality, and the second direct reason of death for children under the age of five years. Average global incidence of PB is estimated to be 11.1%, and there are differences in incidence of PB among different countries and regions – in Africa, for instance, it could be up to 15%, but in some European countries it might be as low as 5–6%. In recent years, with the large-scale application of assisted reproductive technologies and the increase in the number of mothers of advanced age, the incidence of PB is also rising [2, 3].

Preterm infants can experience serious complications, such as respiratory distress syndrome (RDS), sepsis, patent ductus arteriosus, cerebral palsy, and cognitive defects [4, 5]. Approximately 3.1 million neonatal deaths occur worldwide each year, 35% of which are due to prematurity-related complications [6]. Global statistics showed that more than 60% of PB occurred in South Asia and Africa, while incidences of PB differ largely in Asia, which was the lowest in East Asia, with about 7.2%, followed by that in West Asia, with about 10.1%, and the rate was the highest in Southeast Asia, with about 13.6% [7]. There has been no incidence of PB report at the national level for China, and the estimated incidence of PB is below the global level. Furthermore, China has large geographic span, so regional incidences of PB also differ; for example, in Jiangsu Province, the incidence was reported as 2.6–2.9% [8, 9]. This study retrospectively analyzed the clinical data collected from two tertiary referral hospitals, aiming to explore the conditions of PB, remedy levels, and outcomes of preterm infants in this region, thus hoping to provide evidence for the diagnosis and treatment of PB.

The aim of this study was to analyze the cause, clinical features, and outcomes of preterm infants.

METHODS

Data sources

Three hundred and seven puerperae were enrolled, hospitalized in the Yantai Affiliated Hospital of Binzhou Medical University and the Yuhuangding Affiliated Hospital of Qingdao University from January 1, 2012 to December 31, 2012 and pretermly delivered with gestational age of $28–36^{+6}$ weeks. This study was conducted in accordance with the declaration of Helsinki and with approval from

Received • Примљено: August 18, 2016
Revised • Ревизија: April 22, 2017
Accepted • Прихваћено: April 23, 2017
Online first: May 9, 2017

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the ethics committees of Binzhou Medical University and Qingdao University. Written informed consent was obtained from all participants.

Study methods and diagnostic criteria

A retrospective analysis was performed, and PB records that met the criteria were inspected, including general information of pregnant women, pregnancy complications, delivery mode, gestational age at delivery, preterm outcomes (including both neonates and parturient women), as well as the therapy and the course. Meanwhile, the Z-score of neonatal birth weight was also recorded, which depends on the birth weight, Z-score = (birth weight of the infant - mean birth weight with the same number of gestational weeks [GW]) / standard deviation of the birth weight with the same number of GW. The evaluation criteria referred to the standards of neonatal physical development with different number of GW in 15 Chinese cities, and the normal range was usually considered to be within ± 2.

PB was defined as delivery within 28–36^{+6} GW. Classification (three categories according to the reasons) is as follows: spontaneous PB, preterm prelabour rupture of the membrane (PPROM), and iatrogenic PB.

Statistical methods

SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis; the qualitative data were analyzed by the χ^2 test, and the quantitative data were analyzed by the Student's t-test, with $p < 0.05$ considered to be statistically significant.

RESULTS

The average age of women with PB was 30.09 ± 4.45 years, and the mean number of pregnancies was 2 ± 1.29, including 257 primipara (83.71%) and 50 multipara (16.29%), as shown in Table 1.

Causes of PB

The age of pregnant women with iatrogenic PB was 31.26 ± 4.98 years, higher than that of the PPROM group ($p < 0.05$), but not different than that of spontaneous PB ($p > 0.05$). The vaginal delivery rates of spontaneous PB and the PPROM were higher than the iatrogenic PB ($p < 0.05$), as shown in Table 2.

The average gestational age on admission of the 307 cases with PB was 34.13 ± 2.31 weeks, the hospitalization time for tocolysis was 4.13 ± 7.69 days, and the gestational age at delivery was 34.7 ± 2.02 weeks, in which gestational age on admission of the iatrogenic PB was less than that of PPROM ($p < 0.05$). There was no significant difference in the gestational age at delivery among the three groups ($p > 0.05$). The duration for tocolysis of the spontaneous PB was less than the iatrogenic PB but longer than that of PPROM ($p < 0.05$) (Table 3).

Outcomes of preterm infants

The 307 cases with PB delivered a total of 367 neonates, including 56 cases of twin pregnancy (both live birth), three cases of twin pregnancy while one fetus died in utero, and two cases of triplet pregnancy (both live birth). Eight neonates had congenital malformations, including three cases of hypospadias, one of anal atresia, one of congenital esophageal stenosis, one of complete endocardial cushion defect, one of congenital choanal atresia, and one of omphalocele (treated surgically). Among the 367 newborns, neonatal asphyxia occurred in 32, including 11 cases of severe asphyxia, and six newborns died within 30 minutes

Table 1. Comparison of pregnancies and deliveries with different number of gestation weeks (GW)

Parameter	Group 1 28–31^{+6} weeks (n = 25)	Group 2 32–33^{+6} weeks (n = 58)	Group 3 34–36^{+6} weeks (n = 224)
Age (years)	30.52 ± 5.88	30.50 ± 4.57	29.91 ± 4.25
GW on admission	29.67 ± 1.54	32.44 ± 1.19	35.17 ± 1.39
Pre-pregnancy BMI	23.45 ± 3.31	22.98 ± 3.78	22.69 ± 3.72
Time for tocolysis (days)	5.48 ± 10.08	4.88 ± 7.53	3.81 ± 7.50
Delivery mode			
Cephalic vaginal delivery	14 (56%)	19 (32.76%)	86 (38.39%)
C-section	9 (36%)	38 (65.52%)	134 (59.82%)
Breech vaginal delivery	2 (8%)	1 (1.72%)	4 (1.79%)
Number of newborns	31	67	269

BMI – body mass index;
Comparison between GW 28–31^{+6} and GW 34–36^{+6} showed a significant difference, $p < 0.05$;
Comparison between GW 28–31^{+6} and GW 32–33^{+6} showed a significant difference, $p < 0.05$;
Comparison between GW 32–33^{+6} and GW 34–36^{+6} showed a significant difference, $p < 0.05$

Table 2. Short-term outcome of neonates in different GW groups

Parameter	Group 1 28–31^{+6} weeks (n = 25)	Group 2 32–33^{+6} weeks (n = 58)	Group 3 34–36^{+6} weeks (n = 224)
Birth weight (g)	1,483.23 ± 418.47	1,963.88 ± 418.72	2,562.42 ± 488.31
Z-score of birth weight	-0.54 ± 0.89	-0.28 ± 0.93	-0.08 ± 1.12
Number of the newborns	31	67	269
1-minute Apgar			
≤ 3 points	7 (22.58%)	3 (4.48%)	1 (0.37%)
≤ 7 points	3 (9.68%)	8 (11.94%)	10 (3.72%)
Number of newborns transferred to NICU	25 (80.65%)	64 (95.52%)	99 (36.80%)
Pediatric hospitalization (days)	26.86 ± 22.07	16.57 ± 9.29	10.67 ± 6.29
Ventilator use	19 (61.29%)	20 (29.85%)	18 (6.69%)

NICU – neonatal intensive care unit;
Comparison between GW 28–31^{+6} and GW 34–36^{+6} showed a significant difference, $p < 0.05$;
Comparison between GW 28–31^{+6} and GW 32–33^{+6} showed a significant difference, $p < 0.05$;
Comparison between GW 32–33^{+6} and GW 34–36^{+6} showed a significant difference, $p < 0.05$

after birth; a total of 188 neonates were referred to a neonatal intensive care unit (NICU).

Birth weight and Z-score of birth weight for PB neonates

The birth weight of PB neonates was 2,340.46 ± 606.26 g; the birth weight of single-pregnancy neonates was 2,432.77 ± 602.23 g, and that of twin-pregnancy neonates was 2,148.55 ± 565.10, showing no significant difference between these two groups ($p > 0.05$). The average Z-score of the birth weight of PB neonates was -0.15 ± 1.08). The birth weights gradually increased with gestational weeks, and there were significant differences among PB with 28–31^{+6} weeks, 32–33^{+6} weeks, and 34–36^{+6} weeks ($p < 0.05$); Z-score of PB with 28–31^{+6} weeks was lower than that of neonates with 32–33^{+6} weeks and with 34–36^{+6} weeks ($p < 0.05$), but Z-score of neonates between 32–33^{+6} weeks and 34–36^{+6} weeks showed no significant difference ($p > 0.05$) (Table 2). The birth weights and Z-scores of spontaneous PB and PPROM were higher than those of the iatrogenic PB group ($p < 0.05$). The birth weight of the neonates without glucocorticoids administration was higher than of those with glucocorticoids administration ($p < 0.05$), but the Z-scores among those with glucocorticoids administered in less than one course, one course, and none showed no significant difference ($p > 0.05$), as shown in Table 4.

Hospitalized treatment of preterm neonates

Among the 188 neonates with PB referred to NICU, three died of ineffective rescue, and the average hospital stay was 15.17 ± 12.35 days. There was no significant difference in hospital stay among the spontaneous PB, PPROM, and iatrogenic PB groups, but the hospital stay of neonates with delivery less than 32 weeks was longer than that of neonates with 32–36^{+6} weeks ($p < 0.05$), as shown in Table 3 and Table 4.

Ventilator was applied in 57 PB neonates, accounting for 30.32% of those transferred to NICU; the duration of ventilator usage was 5.09 ± 6.91 days. Pulmonary surfactant was administrated in 19 preterm neonates once, and another three infants received pulmonary surfactant twice.

There were 60 cases diagnosed with other diseases: 27 cases of RDS (13.92%), six cases of hypoxic-ischemic encephalopathy (3.09%), two cases of intracranial hemorrhage (1.03%), one case of gastrointestinal bleeding (0.52%), one case of necrotizing enterocolitis (0.52%), two cases of gastroesophageal reflux (1.03%), seven cases of congenital heart disease (3.61%), three cases of shock (1.55%), one case of persistent pulmonary hypertension (0.52%), five cases of disseminated intravascular coagulation (2.58%), two cases of anemia (1.03%), and one case of retinal dysplasia (0.52%).

Table 3. Comparison of pregnancy and delivery with different preterm birth reasons

Parameter	SPB (n = 66)	PPROM (n = 143)	TPB (n = 98)
Age (years)	30.01 ± 4.27	29.32 ± 3.99	31.26 ± 4.98
GW on admission	34.04 ± 2.87	34.55 ± 2.08	33.58 ± 2.09
Pre-pregnancy BMI	21.52 ± 3.38	22.92 ± 3.75	23.71 ± 3.62
GW at delivery	34.66 ± 2.58	34.84 ± 1.86	34.53 ± 1.80
Time for tocolysis (days)	4.46 ± 8.54	2.01 ± 4.40	7.05 ± 9.71
Delivery mode			
Cephalic vaginal delivery	32 (48.48%)	85 (59.03%)	3 (3.06%)
C-section	32 (48.48%)	54 (37.50%)	95 (96.94%)
Breech vaginal delivery	2 (3.04%)	5 (3.47%)	0

SPB – spontaneous preterm birth; PPROM – prelabour rupture of membranes; TPB – therapeutic preterm birth; GW – gestation weeks
Comparison between the SPB group and the PPROM group showed a significant difference, $p < 0.05$;
Comparison between the TPB group and the PPROM group showed a significant difference, $p < 0.05$;
Comparison between the SPB group and the TPB group showed a significant difference, $p < 0.05$

DISCUSSION

Incidence of PB

In recent years, the incidence of PB has been increased mainly due to the popularity of assisted reproductive technologies and the increase in the number of mothers of advanced age [8]; however, incidences of PB reported all over the world, including Asia, are not consistent. For example, 7% in the UK, 13% in India, and more than 15% in Indonesia, Pakistan, etc. [1]. China has a large geographical span; therefore, variation of its PB incidence is also large. An investigation targeting 14 Chinese provinces and cities reported the incidence of PB as 7.04% [10].

Table 4. Short-term outcome of neonates for different preterm birth reasons

Parameter	No hormone treatment (n = 157)	Less than one course of hormone treatment (n = 69)	One course of hormone treatment (n = 81)
Birth weight (g)	2,504.92 ± 594.96	2,185.69 ± 607.74	2,146.85 ± 538.43
Z-score of birth weight	-0.24 ± 1.07	-0.19 ± 1.06	-0.12 ± 1.11
Number of the newborns	193	78	96
1-minute Apgar			
≤ 3 points	4 (2.07%)	4 (5.13%)	3 (3.13%)
≤ 7 points	11 (7.01%)	4 (5.13%)	4 (4.17%)
Number of newborns transferred to NICU	64 (33.16%)	52 (66.67%)	72 (75%)
Pediatric hospitalization (days)	16.03 ± 15.24	15.81 ± 12.22	13.50 ± 8.52
Ventilator use	24 (12.44%)	16 (20.51%)	17 (17.71%)

Comparison between the non-hormone group and the < 1-course hormone group showed a significant difference, $p < 0.05$;
Comparison between the non-hormone group and the 1-course hormone group showed a significant difference, $p < 0.05$;
Comparison between the < 1-course hormone group and the 1-course hormone group showed a significant difference, $p < 0.05$

Furthermore, it showed that the incidence of PB was the highest in southwest regions of China (10.3%), but lowest in central regions (2.3%), and 6% in eastern coastal areas [11]. Another report from Jiangsu province showed that its incidence was low, about 2.6–2.9% [12]. Because the lower limit of gestational age defined for PB in China is 28 weeks, those delivered before 28 weeks are not included in the scope of PB, so there are certain differences between Chinese and international information on PB. The two hospitals included in this study were both tertiary hospitals in Yantai district, as well as the upper-level referral hospitals, and the incidence of PB was reported as 6.58%, similar to the national level.

Causes of PB

Many reports considered PPROM to be a common type of PB, and more than 40% of PB were caused by PPROM; PPROM accounted for 2–4% of singleton pregnancies and 7–20% of twin pregnancies [4, 13]. There is still some controversy about the optimal gestational age of delivery for PPROM, and the American Congress of Obstetricians and Gynecologists once recommended it to be more than 34^{+6} weeks [14]. In this study, PPROM was the most common type, accounting for 46.58 % of PB and 3.06% of total deliveries; the gestational age at delivery was 34.84 ± 1.86 weeks, where delivery time of more than 34^{+6} weeks accounted for 68.75%; the tocolysis time was significantly shorter than in the spontaneous PB and iatrogenic PB groups. Therefore, PPROM should be actively prevented before 37 GW, and attempts should be made to postpone the delivery to after 34^{+6} GW.

In recent years, an important reason leading to the increased incidence of PB is the increase of iatrogenic PB, and it was reported to account for 35–40% of all PB cases in other countries [5]. In this study, the incidence of iatrogenic PB accounted for 31.61%, similar to the reports abroad. The increasing of iatrogenic PB is related with the improvements of medical care level, which makes many pregnancy conditions that were not suitable for pregnancy in the past maintain after 28 GW. In this study, the pregnant women in the iatrogenic PB group were older, had less GW on admission and longer tocolysis time, which all indicated that patients' own diseases had significant impacts on PB.

The reasons of iatrogenic PB could be further divided into three categories. One is caused by maternal diseases, such as intrauterine infection, chorioamnionitis, preeclampsia, or other maternal system disorders; the second is caused by fetal diseases, such as fetal distress or fetal growth restriction; the third is caused by placental diseases, such as placenta previa, or placental abruption [9]. This study showed that the most important reason of iatrogenic PB was pregnancy-induced hypertension (57.14%), similar to studies in Australia and Beijing [15]. The second reason in this study was the prenatal placental factors, such as placenta previa and placental abruption, accounting for 28.57%, which was associated with the increased gravidity and parity, especially in patients with a history of Cesarean section [16]. Therefore, the study suggests that the maternal management of late pregnancy in our region should be focused on the prevention and treatment of the occurrence and progression of pregnancy-induced hypertension.

Analysis of birth weight of preterm infants

Birth weight of preterm infants is closely related to their mortality. In 2010, the mortality rate of live infants in the USA was 6.14‰, in which PB accounted for 35.2%; the mortality of neonates with birth weight less than 1,500 g was 100-fold greater than that of neonates with birth weight greater than 2,500 g [17]. This study introduced the Z-score of birth weight, and the results showed that the Z-score of preterm infants with $28–31^{+6}$ GW was lower than in those with more than 32 GW, while those with $32–33^{+6}$ GW and more than 34 GW showed no significant difference, indicating that gestational age less than 32 GW had a greater impact on fetal body weight in this region, higher than the national average, so nutritional support treatments should be strengthened to increase the fetal body weight in utero.

The study also showed that the birth weight of non-glucocorticoid administration group was higher than the other two groups with glucocorticoid therapy, but there was no significant difference in the Z-score among these groups. It might be so because the gestational age on admission of the non-glucocorticoid group was higher than the other two groups with glucocorticoid therapy, so after eliminating the influence of gestational age, no more difference could be displayed among the treatment groups, indicating that glucocorticoid therapy within one course would not affect fetal birth weight.

Short-term outcomes of preterm infants

Due to immature organ development, complications in preterm infants are numerous [18]. In developed countries, the survival rate of preterm infants with 24 GW was about 50%, and of those with 28 GW it was 90%; however, in developing countries, the survival rate of infants with 28 GW was less than 10%, which could increase to more than 50% only after gestational age reached 34 GW. In 2011, Chinese large-sample data analysis showed the average mortality of preterm infants to be 3.3% [11]. In this study, it was 0.82%, lower than the national average.

Respiratory complications are one type of the most common complications in preterm infants, as well as the main cause of death [19, 20]. RDS in preterm infants is caused by the deficiency of pulmonary surfactant [21]. The incidence of RDS among those with gestational age less than 28 GW accounted for up to 93% [18]. Among the survived preterm infants, approximately 40% would suffer bronchopulmonary dysplasia. When gestational age of preterm infants is increased by one week, or birth weight is increased by 100 g, the risk OR values of bronchopulmonary dysplasia would be 0.77 and 0.89, respectively; therefore, gestational age and birth weight are the key factors affecting the outcome of preterm infants. In this study, the most common complication was RDS, accounting for 13.92%.

Another serious complication in preterm infants is necrotizing enterocolitis, and it was reported abroad to be about 7–11% in preterm infants with birth weight < 1,500 g [22]. In this study, necrotizing enterocolitis occurred in one male preterm infant (delivered at 31^{+1} GW, PPROM, birth weight of 1,830 g, and hospital stay of 35 days), and was discharged when the body weight was increased to 1,920 g. In addition, attention should also be paid to nervous system complications in preterm infants, such as hypoxic-ischemic encephalopathy, intracranial hemorrhage, retinal development, etc. Perinatal infection and maternofetal inflammation caused by different viruses is strongly associated with PB, which represents an important mechanism for cerebral damage.

In short, the main causes of PB, such as PPROM, should be actively prevented and treated during the entire course of pregnancy; at the same time, health care during a pregnancy needs to be strengthened to actively prevent and treat iatrogenic PB, such as pregnancy-induced hypertensive disorders. Establishing effective intrauterine transportation system and actively managing respiratory complications could improve the outcome of PB infants. In this study, there were still some shortcomings; for example, the long-term outcome data of these preterm infants, such as neural development monitoring, were lacking. Therefore, if a perinatal information-sharing network among all the delivery units could be formed in the future, and if doctors in departments of obstetrics and pediatrics could systematically co-survey the outcomes of preterm infants, more accurate data of PB in this region, and even throughout China, would be provided, thus enabling better guidance for obstetricians towards the diagnosis and treatment of PB.

CONCLUSION

The most common reason for PB is iatrogenic, especially maternal diseases. In this study, the birth weight of preterm neonates was less than normal, so Z-score was more adequate to define organ maturity; therapy with glucocorticoids within one course did not affect fetal birth weight. At the same time, since PB could cause a variety of serious complications in infants (such as PPROM), avoiding them based on pre-pregnancy physical examination, pregnancy monitoring, and proper treatment is of utmost importance; preterm infants should also be actively treated so as to improve their outcomes.

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Узроци и непосредни исход код превремено рођене новорођенчади

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САЖЕТАК

Увод/Циљ Превремени порођај (ПП) главни је узрок смртности новорођенчади, а други непосредни узрок смрти до пете године живота детета.

Циљ овог рада је био да анализира клинички ток и исход код превремено рођене деце.

Методе Ретроспективно су анализирани подаци за 307 превремено рођене деце у болници Универзитета у Ћингдау, у периоду од 1. јануара 2012. до 31. децембра 2012.

Резултати Учесталост превременог рађања деце је 6,52%. Превремено пуцање водењака је био разлог у 46,58% ПП-а, спонтани ПП код 66 (21,5%), а терпијски ПП код 98 (31,92%) болесника. Природни порођај је чешћи (60,72%) код трудноћа са < 32 недеље, а чешћи царским резом (60,99%) код трудноћа са ≥ 32 недеље ($p < 0,05$). Порођајна тежина је била 2.340,46 ± 606,26 *g*, а 3-скор на рођењу -0,15 ± 1,08. 3-скор је био мањи у групи 28–31^{+6} недеља него у групи 32–33^{+6} и групи ≥ 34 недеље ($p < 0,05$). Просечна хоспитализација износила је 15,17 ± 12,35 дана, а најчешћа компликација је била респираторни дистрес синдром (13,92%).

Закључак Превремени порођај доводи до озбиљних компликација код новорођенчета. Главни разлози, као прерано пуцање водењака, морају се спречити и лечити, а сáмо новорођенче мора се интензивно лечити.

Кључне речи: одојче; исход трудноће; превремени порођај; 3-скор

DOI: https://doi.org/10.2298/SARH170301088P
UDC: 616.12-007.1-053.2

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Spontaneous closure of muscular ventricular septal defects

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SUMMARY

Introduction/Objective Ventricular septal defect (VSD) is the most frequently diagnosed congenital heart anomaly. The prognosis is usually good as it has spontaneous closure evolution, especially small muscular VSDs.

The aim of this study was to determine the natural history of isolated muscular VSDs including the frequency of spontaneous closure in relation to location in the muscular septum and the age at the time of closure.

Methods The study included 96 children (52 girls and 44 boys) with isolated muscular VSD diagnosed during the first month of life. We analyzed the tendency of spontaneous closure of these defects for the duration of childhood during a follow-up period of 16 years. Two-dimensional color Doppler echocardiography was performed to detect muscular VSD as a primary cardiac lesion. There was significant prevalence of small apical versus trabecular defects and their outcomes were evaluated.

Results Our study evaluated 91 children, 49 (53.8%) girls and 42 (46.2%) boys who did not undergo surgery. Apically located VSD was diagnosed in 68 (74.7%), while trabecular defects were found in 23 (25.3%) children. Spontaneous closure occurred in 56 out of 91 cases (61.5%). The time of spontaneous closure was most commonly recorded during the first six months after birth (46.4%). The overall rate of spontaneous closure was 81.3% by the end of the first year. Apically located ventricular defects underwent spontaneous closure in the majority of patients, in comparison to trabecular ventricular defects ($\chi^2 = 12.581$; $p < 0.001$). Kaplan–Meier analysis demonstrated a significant difference in the average time required for spontaneous closure between the analyzed patient groups (log-rank = 9.64, $p = 0.002$).

Conclusion The frequency of spontaneous closure of muscular VSDs, especially apically located, is very high in the first six months, especially within the first year of life. It is advisable to detect them early on using color flow imaging and to follow up patients up to spontaneous closure.

Keywords: muscular ventricular septal defect; spontaneous closure; color Doppler echocardiography

INTRODUCTION

Ventricular septal defect (VSD) is the most common congenital cardiac anomaly which accounts for up to 42% of cardiac defects [1]. An isolated VSD is defined as a defect in the inter-ventricular septum without other sonographic abnormalities. Isolated VSD occurs in approximately 2–6 of every 1,000 live births, 1.5–3.5 per 1,000 term infants, and 4.5–7 per 1,000 premature infants. VSDs are slightly more common in females, with 46% of cases occurring in males, and 54% in females [2].

Since the 1980s, real-time two-dimensional echocardiography has dramatically improved the noninvasive anatomical assessment of VSD. Cross sectional echocardiography coupled with Doppler echocardiography and color flow imaging are the gold standard for determining the size and location of virtually all VSDs [3]. In muscular septal defect, all views that image the ventricular septum must be employed to detect the defect. Color Doppler echocardiography is critical to determine small asymptomatic defects [4].

The evolution of VSD has been the focus of several studies. Natural history has a wide spectrum, ranging from spontaneous closure to congestive cardiac failure and death in early infancy. Spontaneous closure of VSD especially in the first years of life is a well-known phenomenon and it occurs in about one third of all cases [5]. Closure is most frequently observed in muscular defects (80%), particularly apical, followed by perimembranous defects (35–40%) [6].

We followed up all patients with a muscular VSD diagnosed in the first month of life for over 16 years, to determine the frequency of spontaneous closure in relation to size, location in the muscular septum, and the age at the time of closure.

METHODS

The study included 96 children (52 girls and 44 boys) with isolated muscular VSD diagnosed during the first month of life. We analyzed the tendency of spontaneous closure of these defects throughout childhood during a follow-up period of 1–16 years (from January 2000 to Octobar 2015).

All the patients had complete medical history and were examined by a pediatric cardi-

Received • Примљено: March 1, 2017
Accepted • Прихваћено: March 17, 2017
Online first: March 28, 2017

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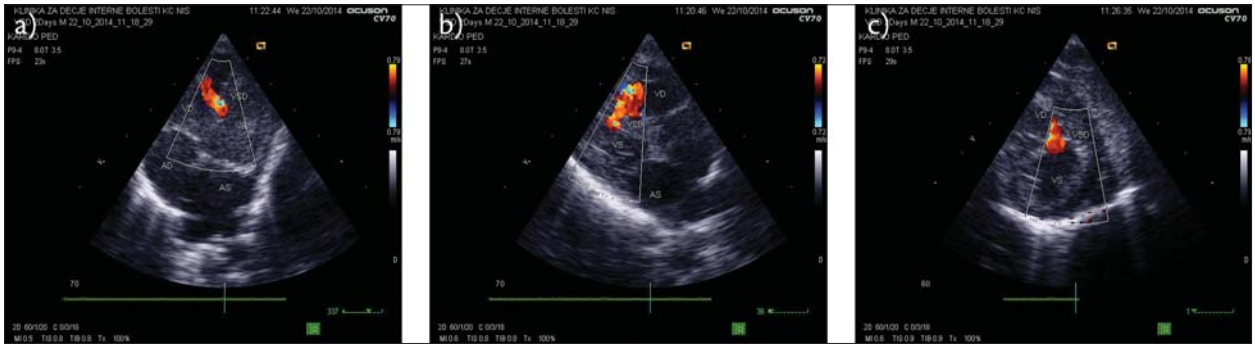

Figure 1. Trabecular muscular ventricular septal defect in a neonate; a) apical four-chamber view; b) parasternal long-axis view; c) parasternal short-axis view

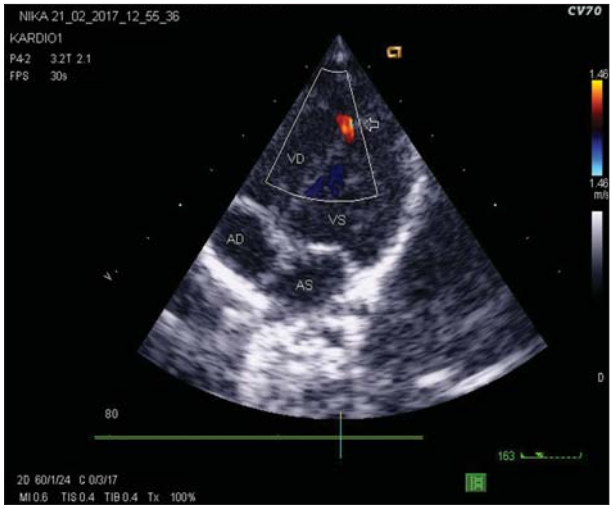

Figure 2. Small apical muscular ventricular septal defect

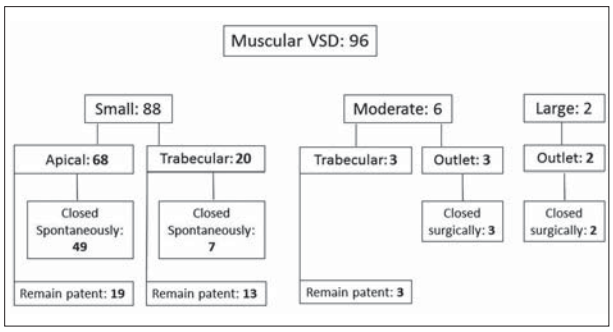

Figure 3. The natural history of the 96 muscular ventricular septal defects (VSDs) studied

ologist. Two-dimensional color Doppler echocardiography was performed using available equipment (HP Image Point – Hewlett Packard Company, Palo Alto, CA, USA, and Acuson CV70 – Siemens Healthineers, Erlangen, Germany) to detect muscular VSD as a primary cardiac lesion. Two subcostal views, parasternal long and short axis, and apical four chamber views were routinely performed to clearly check the complete ventricular septum (Figure 1). When color imaging showed inter-ventricular shunting, the diagnosis was confirmed by color Doppler flow mapping, which indicated velocity and direction of the flow.

The muscular defects were categorized as apical, trabecular, or outlet, according to the classification of Gatzoulis et al. [7]. Defect sizes were measured in two-dimensional images or as the maximum thickness of a color jet at the level of ventricular septum. VSDs were deemed to be large if the defects were as large as or greater than half of the aortic orifice, and small if only seen in some parts of the cardiac cycle or not seen at all, but identified on color flow mapping (Figure 2). All other defects were classified as moderate.

The patients were followed up at approximately three, six, nine, and 12 months of age. After the first year, follow-up intervals were six months. All the patients received prophylaxis for infective endocarditis.

The χ^2 test was used to compare the difference between the prevalence of VSDs in males and females. Actuarial event-free curves were obtained using Kaplan–Meier analysis to compare spontaneous closure rates of apical and trabecular VSDs. Log-rank analysis was used to examine the significance between the curves for apical and trabecular VSD. A p-value of less than 0.05 was regarded as statistically significant.

RESULTS

The study included 96 children (52 girls and 44 boys) with isolated muscular VSD. With regard to size, 88 defects were characterized as small, six as moderate, and two as large defects. According to localization, in the small category there were 68 apical and 20 trabecular; among moderate defects there were three trabecular and three outlet, and in the category of large defects there were two outlet defects. Five outlet defects required surgical closure, three of moderate size and two large ones before the third year of life. Of the 91 patients managed non-surgically, 56 muscular defects had spontaneously closed: 49 were apical and seven were trabecular. Of 35 muscular VSDs that did not require surgical closure and remain open, 19 were apical and 16 were trabecular. Figure 3 summarizes the natural history of 96 muscular VSDs.

Our study evaluated 91 children, 49 (53.8%) girls and 42 (46.2%) boys who did not undergo surgery.

Apically located VSD was diagnosed in 68 (74.7%) patients, while trabecular defects were found in 23 (25.3%) children (Table 1). With regard to defect type, the difference in frequency between girls and boys was not statistically significant ($\chi^2 = 0.089$; $p = 0.766$).

Table 1. Defect type according to sex

Sex	Apical VSD	Trabecular VSD	χ^2	p
Girls	36 (52.9)	13 (56.5)	0.089	0.766
Boys	32 (47.1)	10 (43.5)		

Table 2. Time of spontaneous closure during follow-up period

Time of the spontaneous closure (months)	Number of patients	Ratio (%)	Cumulative ratio (%)
≤ 1	4	7.1	7.1
1 – ≤ 3	13	23.3	23.4
3 – ≤ 6	26	46.4	76.8
6 – ≤ 12	7	12.5	89.3
12 – ≤ 18	4	7.1	96.4
18 – ≤ 42	1	1.8	98.2
42 – ≤ 120	1	1.8	100
Total	56	100	-

Table 3. Defect type according to the outcome of the closure

Parameter	Apical VSD	Trabecular VSD	χ^2	p
No closure	19 (27.9)	16 (69.6)	12.581	< 0.001
Closure	49 (72.1)	7 (30.4)		

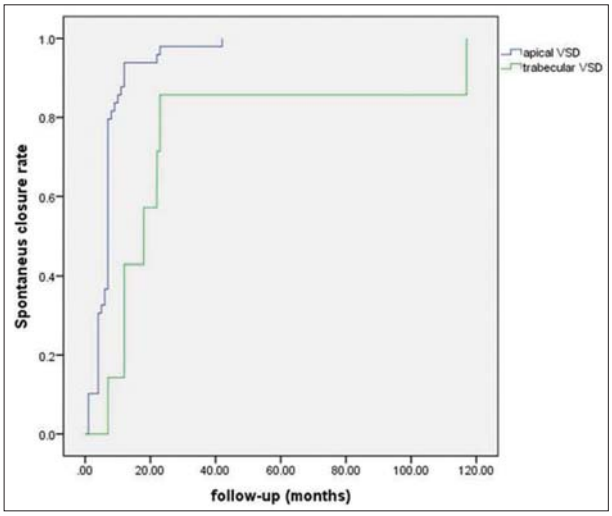

Figure 4. Kaplan–Meier survival curves comparing spontaneous closure rates of apical and trabecular ventricular septal defects (VSDs)

Spontaneous closure occurred in 56 out of 91 cases (61.5%). The average time required for spontaneous closure was 10.4 ± 16.1 months, median 7 months (minimum 1 month and maximum time 117 months), where patient follow-up lasted for a total of 192 months (16 years). The time of spontaneous closure was most commonly recorded during the first six months after birth. At the sixth month, the first year, and the 18th month, spontaneous closure occurred in 43 (46.4%), 50 (89.3%) and 54 (96.4%) cases, respectively. It was seen in all cases except two within the first 18 months; the other defects closed at the 42nd and 117th month (Table 2).

Apically located ventricular defects underwent spontaneous closure in the majority of patients, in comparison to trabecular ventricular defects ($\chi^2 = 12.581$; $p < 0.001$) (Table 3).

Among children in whom spontaneous VSD closure was confirmed, Kaplan–Maier analysis demonstrated that the average time for closure of apical defects was 7.6 months (95% CI: 5.71–9.39 months), and 30.1 months in children with trabecular defects (95% CI: 1.44–58.84 months). A significant difference was found in the average time required for spontaneous closure between the analyzed patient groups (log-rank = 9.64, p = 0.002) (Figure 4).

There was no record of infective endocarditis in any patients.

DISCUSSION

Spontaneous closure is the main characteristic of the natural history of VSD. It is generally accepted that the prognosis of isolated VSD in the postnatal period is good, with a high rate of spontaneous closure during the first years of life. However, frequency of spontaneous closure varies greatly from one study to another, depending on size and location, the population-age studied and the follow-up period [8]. In previous clinical studies, the rate of spontaneous closure of muscular VSD has been reported between 24% and 96%. These rates are quite different, but as a common result, most of the small defects close within a few months after birth [6, 9]. Some investigators suggested that small defects are not a malformation and that early spontaneous closure of these defects is a normal developmental process [10]. Our results show a high rate of spontaneous closure of muscular defects (61.5%) and a relatively high rate of closure in the first year of life with cumulative ratio of 89.3%. Chang et al. [3] published almost an identical frequency (89.2%) of spontaneous closure of muscular defects in the first year. Similar results were published by Xu et al. [11], who reported that up to 78.5% of the whole spontaneous closure events occurred when patients were under the age of three years.

There are a few clinical reports related to the rate of closure for muscular VSDs, where closure rate is influenced by the location of the defect. The results are very different depending on the study. Turner et al. [9] confirmed that the position of a VSD is extremely relevant to its natural history. The spontaneous closure rate for muscular defects was significantly greater than for perimembranous defects. Shirali et al. [6] studied 156 cases for a mean of 28 months and also found a significantly higher spontaneous closure rate for muscular defects. Ramaciotti et al. [12] reported that the rate of closure for muscular VSDs and apical muscular VSDs was 24% and 23%, respectively. They emphasized that spontaneous closure of muscular VSDs was most commonly seen in the first 18 months of life. They also observed that the natural history of a single muscular VSD is not influenced by the location in the muscular septum. Du et al. [10] screened full-term neonates with color flow Doppler imaging for muscular VSDs. The rate of closure at the end of the first year was found as 84.8%, but only one fourth of defects were located in the apical region. They found that defects localized in the apical region and defects greater than 4 mm in size remain patent more than VSDs located elsewhere. Hiraishi et al. [4] found a very high frequency for isolated VSDs when term neonates were rou-

tinely investigated using echocardiography. Most of the defects were small muscular and 76% of them had closed by the first birthday, but 45% were apical muscular VSDs [4].

Our findings are very similar to the ones reported by Turner et al. [9] and Atalay et al. [13]. In Atalay's study, a very high frequency of spontaneous closure of apical muscular VSDs was found. Spontaneous closure was seen in 24 out of 42 cases (57.1%) between one and 36 months of age, and it was most commonly recorded during the first six months of life.

Spontaneous closure becomes less likely during adolescence and adult life. In the study by Gabriel et al. [14], spontaneous closure occurred in 6% of patients.

In recent years, with the development of echocardiographic techniques, the time of diagnosis and monitoring of VSD focuses to the prenatal period [15]. Li et al. [16] concluded that birth weight and prenatal echocardiographic measurement of size and location of VSD enables the estimation of spontaneous closure probability in individual patients.

CONCLUSION

The high chance of spontaneous closure is one of the major reasons why small VSDs are followed conservatively. However, it is important to detect even a small VSD because of the risk of infective endocarditis. Most muscular defects undergo a complete or substantial spontaneous closure during the 12-month follow-up period. Color Doppler echocardiography is a useful technique for establishing the natural course of VSD even from prenatal period. Because of the high closure rate of muscular VSDs, especially apical, and the absence of serious clinical signs, parental anxiety should be minimized.

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Спонтано затварање мускуларних вентрикуларних септалних дефеката

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САЖЕТАК

Увод/Циљ Вентрикуларни септални дефект (ВСД) најчешћа је урођена срчана мана. Прогноза је у највећем броју случајева добра, посебно ако се ради о малим мишићним дефектима, имајући у виду њихову склоност ка спонтаном затварању.

Циљ рада био је да утврди природну еволуцију изолованих мишићних ВСД-а, односно учесталост спонтаног затварања у зависности од њихове локације у мишићном септуму, као и старости болесника у време затварања.

Методе Испитивање је обухватило 96 деце (52 девојчице и 44 дечака) са изолованим мишићним ВСД-ом, који је дијагностикован током првог месеца живота. Анализирали смо тенденцију спонтаног затварања током детињства, а време праћења износило је 16 година. За дијагнозу мишићног ВСД-а као примарног срчаног дефекта коришћена је дводимензионална колор доплер ехокардиографија. Регистрована је значајно већа учесталост малих апикалних ВСД-а у односу на трабекуларне и анализирана је њихова природна еволуција.

Резултати Студијом је обухваћено 91 дете – 49 (53,8%) девојчица и 42 (46,2%) дечака, који нису оперисани током праћења. Апикални мишићни ВСД дијагностикован је код 68 (74,7%) деце, док је трабекуларни нађен код 23 (25,3%) испитаника. До спонтаног затварања дошло је код 56/91 (61,5%) испитаника. Највећи број дефеката затворио се током првих шест месеци живота (46,4%). Укупна стопа спонтаног затварања до краја прве године била је 81,3%. Затворио се значајно већи број апикалних ВСД-а у поређењу са трабекуларним дефекатима ($\chi^2 = 12,581$; $p < 0,001$). Каплан–Мајерова анализа је показала и значајну разлику у просечном времену спонтаног затварања ове две испитиване групе (Лог Ранк = 9,64, $p = 0,002$).

Закључак Може се закључити да се највећи број спонтаних затварања ВСД-а одвија у првих шест месеци, односно до краја прве године живота. Притом се најчешће затварају апикални мишићни дефекти, те их треба на време откривати и континуирано пратити до њиховог затварања.

Кључне речи: мускуларни вентрикуларни септални дефект; спонтано затварање; колор доплер ехокардиографија

DOI: https://doi.org/10.2298/SARH160629057S
UDC: 611.81

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Anatomical and MRI relations of the cerebral aqueduct to the adjacent parts of the brain and calvaria

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SUMMARY

Introducton/Objective Insufficiency of relevant anatomic data and great neurological and neurosurgical significance were the reasons for this study with scientific and practical implications.
The purpose was to determine, at the transverse in situ section of the head, the position and relations of the sylvian aqueduct of the mesencephalon by measuring its distances from particular brain and calvaria structures. Also, the aim was to determine the same distances according to axial sections by using MRI.
Methods The material consisted of twenty autopsy human heads. The section of the head was made at the level of the tentorial hiatus and the midbrain. After that, we measured the distances between the cerebral aqueduct and a) posterior border of the optic chiasm, b) upper border of the dorsum sellae, c) terminal bifurcation of the basilar artery, d) beginning of the straight sinus, e) internal occipital protuberance, f) tentorial edge (lateral from the aqueduct), and g) internal surface of the calvaria (lateral to the aqueduct). We determined the same distances by the MRI system. The measurements were made in 37 subjects.
Results The numerical data obtained by this study will be of benefit to neurosurgeons in choosing a surgical approach to the contents of the incisural space, and to neurologists for the exact localization of the lesion and interpretation of certain signs and symptoms.
Conclusion The results of a detailed examination of the sylvian aqueduct position and relations have shown that the use of MRI is the morphometric method of choice, because it is more precise for all the parameters monitored than in situ measurements.
Keywords: sylvian aqueduct; measured distances; midbrain section; MRI

INTRODUCTION

The sylvian aqueduct is the central cavity of the midbrain. The cerebral aqueduct was first illustrated by Leonardo da Vinci (1452–1519), who injected the ventricles with a solidifying medium and obtained casts – the first anatomical injection, but it was named after Jacques Dubois (Jacobus Sylvius) (1478–1555), a French anatomist and surgeon from Paris, who is not be confused with Francois de la Boe (1614–1672), known as Sylvius, a professor of medicine in Leyden, known for his descriptions of the brain (sylvian fissure) [1, 2]. According to *Terminologia Anatomica* [3], the aqueduct of the midbrain (aqueductus mesencephali) is also correct, and may be more appropriate recommended term. It lies between the tectum and the tegmentum of the midbrain, connecting the third to the fourth ventricle.

The aqueduct has arcuate shape on the medio-sagittal section of the midbrain [4, 5]. Woollam and Millen [6] have defined its rostral and caudal border. The rostral border is a plane perpendicular to the longitudinal axis of the brainstem, passing through the caudal end of the posterior commissure. The caudal border is a plane, also perpendicular to the longitudinal axis of the brainstem, lying at the level of the lower pole of inferior colliculi of the midbrain. On the frontal section, the aqueduct of Sylvius is of triangular shape, with its apex directed downwards.

The review of comparative anatomy of the mammalian brain shows that the cavity of the midbrain gradually narrows from lower to upper vertebrata; in men, it is reduced to a strip duct, therefore the sylvian aqueduct is the most narrow part of the ventricular system and in many cases the site of the liquor blockade.

Received • Примљено: June 29, 2016
Revised • Ревизија: September 1, 2016
Accepted • Прихваћено: September 12, 2016
Online first: March 7, 2017

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Aqueductal stenosis is most frequently a congenital anomaly occurring as the result of the intrauterine infection or other causes [7]. Progressive stenosis of the sylvian aqueduct initiated during the embryonic period may result from ependymal disruption of the cerebral aqueduct and dysfunction of the subcommissural organ [8]. These childhood forms of stenosis are more dangerous and have been studied more extensively than adult forms, although the latter have not been uncommon [9]. Aqueductal stenosis and the slightest alterations in the position, shape, and size of the aqueduct have serious effects on the function of the midbrain structures, and can lead to different neurologic disturbances (hydrocephalus, spastic paresis, muscular rigidity), and may be accompanied by divergence paralysis [10], hydrocephalus [8, 9], schizophrenia and parkinsonism [11, 12, 13], as well as periaqueductal dysfunction (the sylvian aqueduct syndrome) [14].

The midbrain, and the sylvian aqueduct within it, represents the upper part of the brainstem. It lies along the neuraxis passing through the tentorial hiatus, which means that it is both in the supra- and infratentorial space. Therefore, in expansive changes, movements of the brain mass lead to supra- and infratentorial herniation of the midbrain. In these cases, the midbrain is exposed to the pressure from the prolapsed part of the brain and edges of the tentorium, which affects the sylvian aqueduct [15, 16].

Insufficiency of relevant anatomic data and great neurological and neurosurgical significance were the reasons for this study. The aims of our study were to define the position, extent and relationships of the Sylvian aqueduct using transverse sections of the midbrain.

The purpose of this study was to determine, at the transverse in situ section of the head, the position and relations of the sylvian aqueduct of the mesencephalon by measuring its distances from particular brain and calvaria structures. Also, the aim was to determine the same distances by using magnetic resonance imaging (MRI) according to axial sections in FFE-T1 sequence, the slice thickness being 1.5 mm.

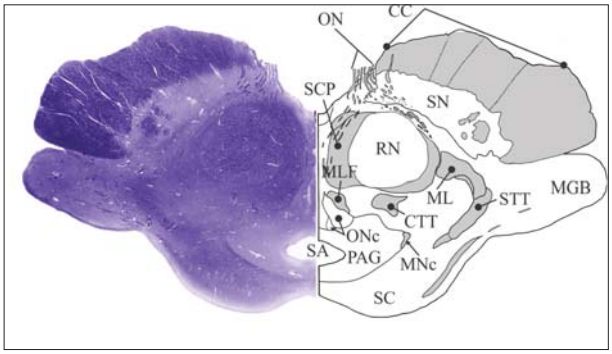

Figure 1. Light microscopy of the transverse section of the midbrain through the superior colliculus (SC), and the red nucleus (RN); SA – Sylvian aqueduct; PAG – periaqueductal grey substance; CC – crus cerebri; SN – substantia nigra; ON – oculomotor nerve; SCP – superior cerebellar peduncle, decussatio; LM – lemniscus medialis; STT – spinothalamic tract; CTT – central tegmental tract; Onc – oculomotor nuclei; MLF – medial longitudinal fasciculus; MNc – mesencephalic nucleus of CN V (cresyl violet and luxol fast blue)

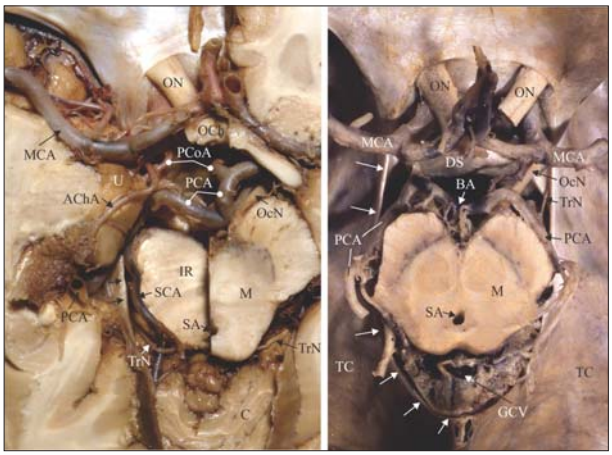

Figure 2. Transverse sections of two heads and midbrains (M) at the level of the tentorial hiatus; SA – sylvian aqueduct; OCh – optic chiasm; ON – optic nerve; IR – isthmus rhombencephali; arrows, tentorial border; TC – tentorium cerebelli; PCA – posterior cerebral artery; PCoA – posterior communicating artery; MCA – middle cerebral artery; AChA – anterior choroidal artery; SCA – superior cerebellar artery; BA – basilar artery; U – uncus; TrN – trochlear nerve; OcN – oculomotor nerve; DS – dorsum sellae; C – cerebellum; GCV – great cerebral vein

METHODS

After a standard preparation procedure for histochemistry, one normal midbrain was embedded in paraffin and sectioned transversely. The slices were stained with cresyl violet and luxol fast blue (Klüver–Barrera method), examined under the light microscope, and photographed using a digital camera (Figure 1). Additionally, we made transverse sections of two formalin injected heads in order to use them for studies of the position, extent, and relationships of the mesencephalon and cerebral aqueduct, as the coronal sections of one formalin injected head (Figures 2 and 3).

Measurements were performed on 20 heads of adults, both sexes, aged between 48 and 65 years (12 female, whose average age was 60 years, and eight male of the average age of 58 years), with no pathological findings, during routine autopsies at the Institute of Pathology, School of Medicine, University of Belgrade. We studied the relationships only in fresh cadaver material with short postmortem delays. After the skull had been opened, the dura mater was removed, and frontal lobes of the cerebrum were lifted. This enabled inspection and detection of the optic chiasm and internal carotid arteries. Then we carried out the sectioning of the midbrain in situ at the level of the tentorial notch. The distances were measured using scientific caliper.

The following distances were measured (Figure 4):

a. from the aqueduct to the posterior border of the optic chiasm;
b. from the aqueduct to the upper border of the dorsum sellae;
c. from the aqueduct to the terminal bifurcation of the basilar artery;
d. from the aqueduct to the beginning of the straight sinus;
e. from the aqueduct to the internal occipital protuberance;

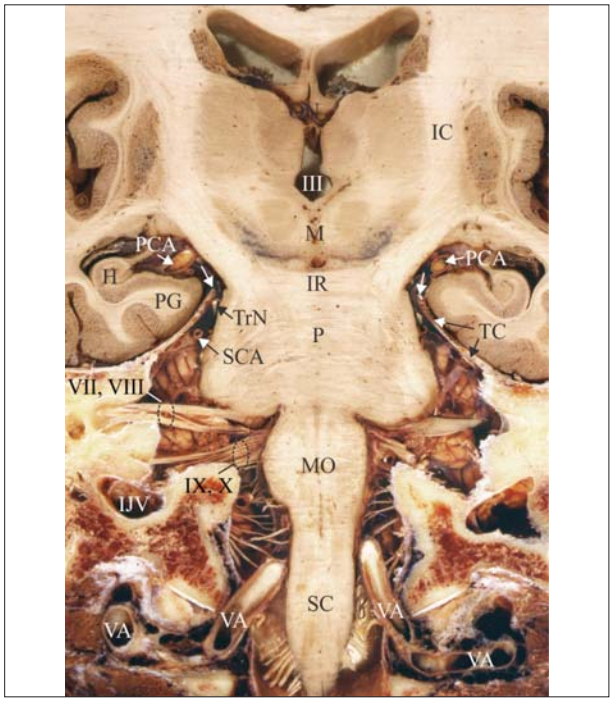

Figure 3. Coronal section of the head through the third ventricle (III); IC – internal capsule; M – midbrain; IR – isthmus rhombencephali; P – pons; MO – medulla oblongata; SC – spinal cord; arrows, tentorial border; TC – tentorium cerebelli; PCA – posterior cerebral artery; H – hippocampus; PG – parahippocampal gyrus; TrN – trochlear nerve; SCA – superior cerebellar artery; VII and VIII – facial and vestibulocochlear nerves; IX and X – glossopharyngeal and vagus nerves; IJV – internal jugular vein; VA – vertebral artery

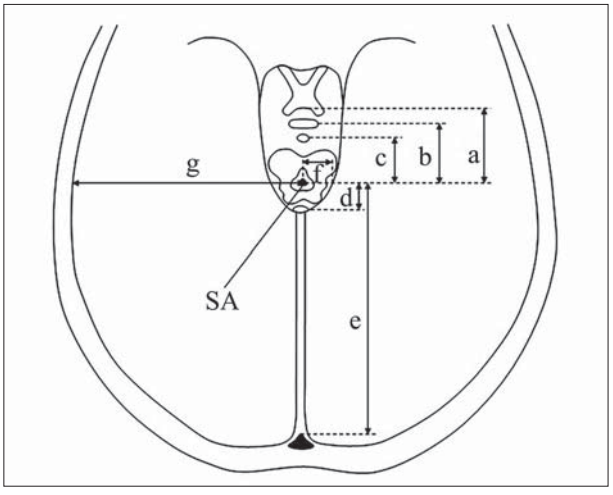

Figure 4. Distances between the sylvian aqueduct (SA) and the: a – posterior border of the optic chiasm; b – upper border of the dorsum sellae; c – basilar artery; d – beginning of the straight sinus; e – internal occipital protuberance; f – tentorial edge; g – internal table of the skull

f. from the aqueduct to the tentorial edge (lateral to the aqueduct), and
g. from the aqueduct to the internal table of the calvaria (lateral to the aqueduct).

We measured the same distances using the Philips Intera 3+ (Koninklijke Philips N.V., Amsterdam, Netherlands) MRI system, using axial sections in the FFE-T1 weighted sequence, TE 15, TR 450, FOV 192 × 240, slice thickness 1.5 mm. The measurements were performed at the Health Centre for Railway Workers, Belgrade, on 37 subjects (20 men of the average age of 44 years, and 17 women of the average age of 41 years), with no pathological findings, after the approval obtained from each person and the institutional ethics committee.

Data obtained by two different methods were compared. Specific matching was performed, and in statistical analysis of data, we used the descriptive statistics (mean value, standard deviation and standard error) implementing the SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA) software. Statistical significance of the differences among the mean values was tested by Student's t-test.

RESULTS

The cerebral aqueduct, surrounded by the periaqueductal grey substance (PAG), separates the tectum from the tegmentum. PAG contains important structures, such as dorsal raphe nucleus, dorsal tegmental nucleus, dorsal longitudinal fasciculus, and some monoaminergic bundles. The midbrain tegmentum, ventral to the cerebral aqueduct, and dorsal to the substantia nigra, contains the trochlear and oculomotor nuclei, the trigeminal mesencephalic nucleus, the red nuclei, and many smaller cell groups, as well as a curved band of somatic sensory fibers. The massive crura cerebri contain descending corticofugal fibers (Figure 1).

In the tentorial hiatus area, there are also other structures: rhombencephalic isthmus, rostral portion of the cerebellum, uncus, and the parahippocampal gyrus, and a large number of cerebral vessels and cranial nerves (Figures 2 and 3). Major arteries in this area include the anterior choroidal artery, posterior cerebral artery, and superior cerebellar artery. The lateral posterior choroidal arteries and thalamogeniculate arteries, side branches of the posterior cerebral artery, course just medial to the free edge of the tentorium. In this region, the internal cerebral veins and the basal vein of Rosenthal unite to form the great cerebral vein of Galen, which joins the inferior sagittal sinus to form the straight sinus. The trochlear nerve passes through this region and is closely related to the tentorial free edge.

We compared anatomical and radiological distances (diameters) to define the exact position of the Sylvian aqueduct. In order to accomplish this we measured distances between the cerebral aqueduct and particular structures: a) posterior border of the optic chiasm, b) upper border of the dorsum sellae, c) terminal bifurcation of the basilar artery, d) beginning of the straight sinus, e) internal occipital protuberance, f) tentorial edge (lateral to the aqueduct), and g) internal table of the calvaria (lateral to the aqueduct) (Figure 4).

a) The average distance between the aqueduct and the posterior border of the optic chiasm was 43.2 mm, SD 3.76, SE 0.84. The minimal value was 40 mm and maximum 48 mm (Table 1A). The average value of the same distance obtained by MRI was 44.6 mm,

Table 1. Statistics of all measured distances in situ and on MRI

Distance from the cerebral aqueduct to:	a. Chiasma opticum	b. Dorsum sellae	c. Basilar artery	d. Straight sinus	e. Internal occipital protuberance	f. Tentotial edge			g. Internal table of calvaria		
						Left	Right	Mean	Left	Right	Mean
A. Measured on sections											
Mean	43.2	36.8	26.2	22.1	63.2	10.8	11.2	11	65.9	66.1	66
SD	3.76	2.40	2.69	3.10	3.22	0.89	0.91	0.89	3.49	3.67	3.49
SE	0.84	0.54	0.6	0.69	0.72	0.2	0.17	0.2	0.78	0.82	0.78
Min.	40	31	23	17	56	9	10	9	57	57	57
Max.	48	41	32	28	71	12	12	12	71	72	72
B. Measured on MRI											
Mean	44.6	36.3	26.3	22.9	61.9	10.5	10.8	10.6	64.1	64.3	64.2
SD	2.33	2.83	2.26	3.71	3.7	0.94	0.96	0.94	4.81	4.78	4.8
SE	0.38	0.47	0.37	0.61	0.61	0.15	0.16	0.16	0.79	0.79	0.79
Min.	40.5	31.5	22.2	17.2	57.2	9.2	9	9.2	57.2	57.8	57.5
Max.	48.1	40.7	31.8	28.9	70.9	12	12.1	12	72.2	72	72.1
DF = 72											
t	1.925	0.820	0.173	1.007	1.612	1.410	1.839	1.880	1.842	1.817	1.845
p	0.058	0.415	0.863	0.318	0.111	0.163	0.070	0.064	0.070	0.073	0.069

SD 2.33, SE 0.38, the lowest value 40.5 mm, and the highest 48.1 mm (Table 1B).

b) The mean value of the distance between the aqueduct and the upper border of the dorsum sellae was 36.8 mm, SD 2.4, SE 0.54, the minimal value was 31 mm, and the maximal 41 mm (Table 1A). The average value of that distance on MRI was 36.3 mm, SD 2.83, SE 0.47, the lowest value 31.5 mm, and the highest 40.7 mm (Table 1B).

c) The mean value of the distance between the aqueduct and the terminal bifurcation of the basilar artery was 26.2 mm, SD 2.69, SE 0.6, the minimal value was 23 mm, and the maximal 32 mm (Table 1A). The average value of the same MRI distance was 26.3 mm, SD 2.26, SE 0.37, the lowest value 22.2 mm and the highest 31.8 mm (Table 1B).

d) The mean value of the distance between the aqueduct and the beginning of the straight sinus was 22.1 mm, SD 3.1, SE 0.69, the minimal value was 17 mm, and the maximal 28 mm (Table 1A). The average value of the same distance obtained by MRI was 22.9 mm, SD 3.71, SE 0.61, the lowest value 17.2 mm and the highest 28.9 mm (Table 1B).

e) The mean value of the distance between the aqueduct (section at the level of hiatus tentorii) and the most prominent point of the internal occipital protuberance was 63.2 mm, SD 3.22, SE 0.72, the minimal value was 56 mm, and the maximal 71 mm (Table 1A). The average value of that distance on MRI was 61.9 mm, SD 3.7 mm, SE 0.61, the lowest value 57.2 mm and the highest 70.9 mm (Table 1B).

f) The mean value of the distance between the aqueduct (section at the level of hiatus tentorii) and the tentorial edge was, on both sides, 11 mm, SD 0.89, SE 0.2, with the minimal value of 9 mm, and the maximal 12 mm (Table 1A). The average value of the same distances obtained by MRI for the left and the right side on average was 10.6 mm, SD 0.94, SE 0.16, the lowest value 9.2 mm, and the highest 12 mm (Table 1B).

g) The mean value of the distance between the aqueduct and the internal table of the calvaria was, on both sides, 66 mm, SD 3.49, SE 0.78, with the minimal value of 57 mm, and the maximal 72 mm (Table 1A). The average value of these distances on MRI for the left and right side was 64.2 mm, SD 4.8, SE 0.79, the lowest value 57.5 mm and the highest 72.1 mm (Table 1B).

Additionally, we compared the accuracy of measurements made using MRI axial sections (Figure 5) with anatomical measurements obtained by using a sliding caliper. There were no statistically significant differences between the MRI and the anatomical measurements (Student's t-test; $p > 0.05$) (Table 1). Our results demonstrated that MRI sections can provide accurate and relevant measurements which are in good correlation with performed anatomical measurements.

The results of a detailed examination of the sylvian aqueduct position and relations have shown that use of MRI is morphometric method of choice, because it is more precise for all the parameters monitored than in situ measurements. The bony tissue is not prone to postmortal alterations, and it has been well known that connective membranes are more resistant, but with increasing post mortem delay softening of nervous tissue occurs. This may result in deformation and significant changes of the brain stem position in the supratentorial and infratentorial compartments.

DISCUSSION

The region of the tentorial notch can be classified as the: anterior, middle (paired), and posterior incisural space. The posterior incisural space is also known as the pineal region. It lies in front of the cerebellum, posterior to the

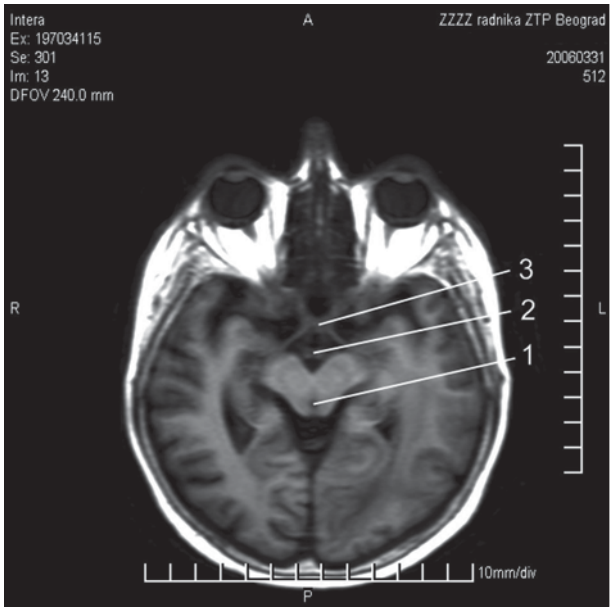

Figure 5. Section of mesencephalon at the level of hiatus tentorii obtained by MRI; 1 – sylvian aqueduct; 2 – basilar artery; 3 – optic chiasm

midbrain, partly situated in the cerebello-mesencephalic fissure. It corresponds to a quadrigeminal cistern, extending superiorly to the lower surface of the splenium, and posteriorly to the level of the tentorial apex. Its lateral borders are the pulvinares of thalami and the posterior part of parahippocampal gyrus, laterally extending to the middle incisural space. The middle incisural space is a narrow space between the upper part of the brainstem medially (at the level of pontomesencephalic juncture) and the medial side of the temporal lobe, more precisely, the parahippocampal gyrus. Superiorly, this space is bounded by the posterior part of the optic tract and the lower surface of the thalamus, and it continues inferiorly into the cerebello-mesencephalic fissure. The middle incisural space corresponds to the ambiens and crural cisterns [15, 16].

Lang and Buhler [17] and Lang [18] presented data on the dimensions of certain midbrain structures: crus cerebri, lamina tecti, tegmentum, superior colliculi, inferior colliculi, interpeduncular fossa, but none of the distances from the aqueduct to surrounding structures. Our measurements, which have not been performed before, are collected and compared with those made by using MRI, and showed, in general, position and relations of the sylvian aqueduct with possible clinical implications.

A specific challenge for a neurosurgeon is the approach to the contents of the incisural space, particularly the lesions along the posterolateral midbrain. In these cases, the adequate surgical approach is supracerebellar and infratentorial [19]. This approach has three variants: middle, paramedian (lateral), and extremely lateral. Therefore, it is important to evaluate the relations of the aqueduct to the tentorial notch, elements of the incisural space, as well as other parts of the brain and calvaria. Neurosurgical significance of the mesencephalic aqueduct and PAG is manifested in appropriate neurosurgical interventions at the mesencephalic level. The most frequent surgeries are in the vicinity of the midbrain, both dorsally (in the pineal body area), and ventrally (in patients with craniopharyngioma). Surgical procedures at the level of the mesencephalon have been rare. Therefore, the knowledge of aqueductal topography is of importance in both fields – neurology and neurosurgery.

Even though neurosurgeons consider the aqueduct an untouchable cerebrospinal fluid channel, with increasing popularity of neuroendoscopy and stereotactic neurosurgery, structures in its vicinity could be managed endoscopically or stereotactically. Longatti et al. [2] described the findings of endoscopic aqueductal exploration. But, the anatomy of the periaqueductal region is extremely complex, and the idea that the aditus can be forced and the aqueduct itself mechanically enlarged with a larger endoscope is certainly hard to accept [20].

Neurological significance of PAG is reflected in appearance of various signs and symptoms in patients with dorsal tegmental lesion (the aqueduct and PAG lesion). Lesions can be of vascular, neoplastic and demyelinating in their natures. Vascular lesions most frequently occur after occlusion of paramedian perforating branches of posterior cerebrale and/or terminal part of the basilar artery [21]. Tumors appear either out of the mesencephalon, for example in the region of the pineal body with compression on the dorsal tegmentum, or in the PAG proper [22, 23, 24]. Finally, demyelinating diseases may affect certain parts of PAG and surrounding formations. For interpretation of mental correlates accompanying structural lesions in the mesencephalic region, it is important to properly interpret alterations and form of mesencephalic aqueduct, its distance from the left and right substantia nigra and dorsal tegmentum, as well as asymmetry of the above elements on MRI scans. The fact is that, namely, substantia nigra, nucleus raphe dorsalis and PAG are responsible for affective functions and emotions, as well as that there coexist different neurotransmitters and neuropeptides with a particular role in modulating mental functions. There are 40% of all GABA cerebral axonal terminals in substantia nigra, representing the most potent inhibitory neurotransmitter in the mammalian central nervous system.

The tentorial hiatus is a frequent site of herniation in cases of intracranial hypertension. The supratentorial expansive processes lead to descending herniation and infratentorial processes to ascending herniation. In both cases there is compression, dislocation, elongation and, eventually, laceration of the cerebral tissue, nerves, arteries, veins, and liquor space. As a result, edema, infarction, hemorrhage, and hydrocephalus develop [15]. Considering the fact that the aqueduct is a very narrow channel, it can easily be narrowed by expansive and other processes, either in or adjacent to the aqueduct, and produce stenosis and hydrocephalus [7, 9, 25]. The aqueduct can be expanded in patients with generalized cerebral atrophy, as well is in head injuries followed by the loss of periaqueductal neuronal and axonal substance. A tumor can develop inside the aqueduct, connected to the intraventricular tumors of the third and fourth ventricle. The tumors of the posterior cranial fossa can displace the aqueduct posteriorly, anteriorly, laterally, or can lead to posterolateral shifting [26].

This study has a both neurosurgical and neurological significance. The data obtained will be of benefit to neurosurgeons in choosing a surgical approach to the contents of the tentorial notch, and to neurologists and neuroradiologists for the exact localization of the lesion and further interpretation of certain signs and symptoms.

CONCLUSIONS

The data obtained during this study may facilitate establishing diagnosis of pathological processes in the central part of the cerebral base, since they contribute to the exact location of pathological processes. They may also assist in interpreting the occurrence of accompanying neurologic and psychiatric patient signs and symptoms.

ACKNOWLEDGEMENT

This work was supported by grant No. 175030 awarded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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Односи Силвијевог канала са околним деловима мозга и лобање мерени анатомски и магнетном резонанцом

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САЖЕТАК

Увод/Циљ Недостатак одговарајућих анатомских података и велики неуролошки и неурохируршки значај су били разлози за покретање ове студије са научним и практичним значајем.

Циљ рада је био да се, на попречном пресеку главе, одреде положај и односи Силвијевог канала средњег мозга мерењем раздаљина до одређених структура мозга и лобање. Такође, циљ је и да се одреде исте раздаљине коришћењем попречних пресека магнетне резонанце (МР) главе.

Методе рада Материјал су чинили пресеци 20 глава добијени током рутинске обдукције. Пресеци главе и мозга су прављени у нивоу зјапа шатора малог мозга и средњег мозга. Мерили смо растојања између Силвијевог канала и а) задње ивице оптичке раскрснице, б) горње ивице лећног дела хипофизне јаме, в) завршне рачве базиларне артерије, г) почетка правог синуса, д) унутрашње потиљачне кврге, ђ) ивице тенторијума (упоље од канала средњег мозга) и е) унутрашње површине крова лобање (упоље од канала средњег мозга). Мерили смо исте раздаљине коришћењем МР. Мерење је обављено на 37 особа.

Резултати Нумерички подаци добијени овом студијом биће од користи неурохирурзима у проналажењу хируршког приступа садржају простора између слободних ивица тенторијума, као и неуролозима за прецизну локализацију лезија и интерпретацију неких знакова и симптома.

Закључак Резултати детаљног проучавања положаја и односа Силвијевог канала показали су да је коришћење МР морфометријска метода избора јер је много прецизније за све посматране параметре од мерења током обдукције.

Кључне речи: Силвијев канал; мерене раздаљине; пресек средњег мозга; МР

DOI: https://doi.org/10.2298/SARH160614056M
UDC: 616.314-053.2(497.115)

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Prevalence, characteristics and severity of hypomineralization of the first permanent molars and incisors in children from the northern part of Kosovo and Metohija

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SUMMARY

Introduction/Objective Molar-incisor hypomnineralization (MIH) is relatively common developmental anomaly characterized by hypomineralized enamel defects in the first permanent molars and incisors. The aim of this study was to determine the prevalence of hypomineralization of the first permanent molars and incisors in children aged eight and 10 years who live in the northern part of Kosovo and Metohija.

Methods The study included 712 respondents, 289 of whom aged eight (40.6%) and 423 of whom aged 10 years (59.4%). Criteria according to Weerheijm were used for diagnosis of hypomineralization and the severity of changes was determined.

Results The frequency of hypomineralized changes in the first permanent molars and incisors of the examined children in this area was 12.2%. It was lower in children aged eight years (10.7%) compared to those aged 10 (13.2%). Demarcated enamel opacity was more common in younger children, whereas both atypical restoration and tooth extraction due to hypomineralization were more common in older children. Mild form is more common in children aged eight years, whereas both severe form and severe form with extracted teeth are more common in children aged 10 years. The results indicate that the first permanent molars were most commonly affected by MIH changes.

Conclusion The percentage of the respondents with MIH changes in the northern part of Kosovo and Metohija, which is 12.2%, is not negligible and points to the necessity of early diagnosis in order to prevent and reduce the complications of the condition by timely prevention and treatment.

Keywords: hypomineralization; molars; incisors; children; prevalence

INTRODUCTION

In addition to dental caries and periodontal disease, developmental tooth disorders have been a problem in dentistry for more than two centuries. In economically developed countries, the problem of dental caries has been solved using targeted prevention programs, and significant results are being achieved in prevention of periodontal disease as well. Thus it has been proved that dental diseases can be prevented and controlled [1].

However, in the last ten years, especially in these countries, with a decrease in incidence of dental caries in children and adolescents, irregularities in the structure of hard dental tissues, which occur as a consequence of disorders at the time of their formation, apposition, mineralization, and maturation, have become more and more common. Special attention has been paid to isolated hypomineralized enamel defects of the first permanent molars and incisors.

During the 1970s, Swedish dentists were the first to point to the changes in the form of hypomineralization of the first permanent molars [2]. In 2001 a Dutch dentist Karin Weerheijm [3] suggested the term of molar–incisor hypomineralization (MIH), hypomineralization of molars and incisors, to describe the clinical findings of hypomineralization of systemic origin of one or more of the first four permanent molars, which may be associated with changes in the maxillary, and less frequently in the permanent mandibular incisors.

Although the definition suggests that the influence of systemic factors is important, no specific systemic cause has been determined. It is the multifactorial etiology that is important, and inadequately explored genetic background in the etiology of MIH as well [4, 5].

Factors mentioned in the literature as possible etiological factors that might cause hypomineralization of molars and incisors are premature birth, low birth weight, twins, ear, nose, and throat infections, respiratory problems, epileptic seizures, hypoxia, disorder of calcium and phosphate metabolism, urinary tract infections, infant exposure to dioxins and biphenyls, childhood illnesses, and chronic diseases. Recent studies have shown that frequent antibiotic use and vitamin D deficiency may affect tooth hypomineralization [6–9].

Received • Примљено: June 14, 2016
Revised • Ревизија: December 6, 2016
Accepted • Прихваћено: December 7, 2016
Online first: March 3, 2017

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Hypomineralization of the first permanent molars and incisors, defined as reduced enamel mineralization, is a clinical condition in which enamel defects range from demarcated opacities to cracked, severely hypomineralized enamel. Defective opaque (porous) enamel is of normal thickness, with a smooth surface and can be whitish, white-yellow or yellow-brown and the defect typically has a clear line between affected and healthy enamel [10].Teeth affected by MIH are susceptible to thermal, mechanical and chemical stimuli. Therefore, oral hygiene is difficult, and these teeth are more prone to cavities, which disturbs the oral health of children, and represents a significant problem in pediatric dentistry [11].

The frequency of this phenomenon varies significantly. Studies in different countries show different results, ranging 2.5–40.2% [12–16].

There are no precise data on the frequency of hypomineralization of the first permanent molars and incisors in the territory of Kosovo and Metohija.

Therefore, the aim of this study was to determine the distribution of hypomineralization of the first permanent molars and incisors in children who live in the northern part of Kosovo and Metohija as well as to assess the characteristics and determine the severity of hypomineralization.

METHODS

The study was approved by the Ethics Committee of the Faculty of Medicine in Kosovska Mitrovica (No. 09-2440 / November 26, 2015), and it was conducted by the researcher who had been previously trained and calibrated for assessing hypomineralization of the first permanent molars and incisors and other developmental enamel defects. Examiner reliability for the accurate diagnosis of MIH was tested on 10% of respondents of the total sample. A high inter-rater diagnostic accuracy of MIH ($\kappa = 0.97$) was received.

Children examinations were carried out at the Department of Pediatric and Preventive Dentistry of the Faculty of Medicine in Kosovska Mitrovica and in school dental offices. Dental mirrors, probes, and common lighting were used to examine children. The probes were used only when necessary, for removing dental plaque.

We examined only children who had written parental consent to participate in the study, and who had previously been fully informed, orally and in writing, about the aims of the study.

A total of 712 schoolchildren, 289 (40.6%) of whom aged eight and 423(59.4%) aged 10, were examined in the following elementary schools: "Sveti Sava" and "Branko Radičević" in Kosovska Mitrovica (393 schoolchildren examined), "Vuk Karadžić" in Zvečan (134 schoolchildren examined), and "Leposavić" (185 schoolchildren examined).

Criteria used in this study and commonly used in the literature are those proposed by Weerheijm et al. [17]. These are the following: demarcated opacity (DO); post-eruptive enamel breakdown (PEB); atypical restoration (AR); extracted molar due to MIH (E-MIH); un-erupted teeth (UT).

For patients with DO, PEB, and AR, we determined the severity of changes. There are three degrees of severity: mild, moderate, and severe form. Mild form of the tooth mineralization disorder is characterized by stained changes in the tooth enamel. Moderate form is characterized by changes in color (white/opaque, yellow or brown) and minimal loss of tooth substance with no need for restoration, or minimally invasive treatment is sufficient to repair defects. Loss of damaged enamel and dentin which require restoration represent severe form.

In cases when there were more defects on one tooth, the most severe change was recorded as valid. Teeth with more than one half of the crown erupted were included in the study while lesions less than 1 mm were not.

Of statistical tests, Student's t-test and χ^2 test were used, and the significance test was performed at the level of probability $p < 0.01$ and $p < 0.001$.

RESULTS

Based on the set aims and with appropriate methodology used in the study, the following results were collected.

Table 1 shows a comparative analysis of respondents by sex and age in relation to the occurrence of hypomineralized changes**.** The frequency of MIH in the examined children in this area was 12.2%. It was found that the percentage of female respondents with MIH was higher than that of males, but with no statistically significant difference ($\chi^2 = 0.69$, $p = 0.4058$). Analysis of MIH prevalence in relation to age has lower values in children aged eight years (10.7%) compared to that in respondents aged 10 (13.2%). The analysis of data shows there is no statistically significant difference ($\chi^2 = 1.01$, $p = 0.314$).

When analyzing individual criteria for MIH, neither the difference in the frequency of hypomineralized changes in the form of demarcated enamel opacities ($\chi^2 = 1.72$, $p = 0.190$) nor in the form of post-eruptive enamel breakdown ($\chi^2 = 1.16$, $p = 0.686$) due to MIH, is statistically significant in relation to age groups of children. An atypical restoration is statistically more common in children aged 10 years

Table 1. Distribution of children by sex and age in relation to the occurrence of molar and incisor hypomineralization (MIH)

MIH	Boys		Girls		8 years		10 years		Total sample	
	n	%	n	%	n	%	n	%	n	%
Presence of MIH	38	11.5	49	12.7	31	10.7	56	13.2	87	12.2
No MIH	291	88.5	334	87.3	258	89.3	367	86.8	625	87.8
Total	329	100	383	100	289	100	423	100	712	100

Table 2. Prevalence of molar and incisor hypomineralization (MIH) per individual criterion

MIH criteria	Children %			Molars %			Incisors %			Teeth with MIH %		
	Years			Years			Years			Years		
	8	10	8 + 10	8	10	8 + 10	8	10	8 + 10	8	10	8 + 10
Demarcated opacity	10.7	12.5	11.8	58.1**	18.9	32.9	100	91.2	94.6	65.4**	29.9	42.7
Post-eruptive enamel breakdown	4.5	6.1	5.5	27.6	30	29.2	0	8.8	5.4	22.8	26.7	25.3
Atypical restoration	1.7	6.4*	4.5	11.4	34.7**	26.4	0	0	0	9.4	29.5**	22.2
Extracted molar due to MIH	0.7	4.5*	2.9	2.9	16.3**	11.5	0	0	0	2.4	13.8**	9.6
Unerupted teeth	0	0	0	0	0	0	0	0	0	0	0	0

*p < 0.01
**p < 0.001)

Table 3. Frequency of severity degrees of molar and incisor hypomineralization (MIH) changes

MIH expression level	Children %			Molars %			Incisors %			Teeth with MIH %		
	Years			Years			Years			Years		
	8	10	8 + 10	8	10	8 + 10	8	10	8 + 10	8	10	8 + 10
Mild form	10.7	12.5	11.8	50.9*	20.1	32.1	100	91.2	94.6	59.7*	32.6	43.2
Moderate form	3.8	4.5	4.2	33.4	34.7	34.1	0	8.8	5.4	27.4	30	29
Severe form	2.4	8*	5.8	15.7	45.2*	33.8	0	0	0	12.9	37.3*	27.8
Severe form (present + extracted teeth)	3.1	12.5*	8.7	18.1	54.2*	41.2	0	0	0	15	46*	34.8

*p < 0.001

(χ^2 = 8.83, p = 0.003) as well as tooth extraction due to MIH (χ^2 = 8.23, p = 0.004).

A demarcated opacity in molars is statistically more common in children aged eight years (χ^2 = 46.96, p < 0.001), whereas both an atypical restoration (χ^2 = 18.89, p < 0.001) and tooth extraction due to MIH (χ^2 =12.01, p < 0.001) are statistically more common in molars in children aged 10 years. There is no statistically significant difference in either frequency of demarcated opacities in incisors (χ^2 = 2.05, p = 0.152) or in post-eruptive enamel breakdown (χ^2 = 2.05, p = 0.152) in relation to age groups of children.

Demarcated opacity in teeth with MIH changes is statistically more common in children aged eight years (χ^2 = 41.61, p < 0.001), whereas both an atypical restoration (χ^2 = 18.79, p < 0.001) and tooth extraction due to MIH (χ^2 =12.20, p < 0.001) are statistically more common in children aged 10 years. Post-eruptive enamel breakdown in all teeth with MIH changes is with no statistically significant difference in relation to age groups (χ^2 = 0.67, p = 0.414) (Table 2).

Frequency of some severity degrees by number of the respondents and affected teeth present in the respondents' mouths is shown in Table 3.

There is no statistically significant difference in either frequency of the mild (χ^2 = 0.54, p = 0.46) or moderate form (χ^2 = 0.20, p = 0.65) in relation to age groups of children. The severe form is statistically more common in children aged 10 years (χ^2 = 11.64, p < 0.001) as well as the severe form with extracted teeth (χ^2 =19.15, p < 0.001).

Mild form in molars is statistically more common in children aged 8 years (χ^2 = 35.47, p < 0.001) whereas both severe form (χ^2 =16.58, p < 0.001) and severe form with extracted teeth (χ^2 =36.37, p < 0.001) are statistically more common in molars in children aged 10 years. There is no statistically significant difference in frequency of moderate form in relation to age groups of children (χ^2 = 0.20, p = 0.65). There is no statistically significant difference in either frequency of mild (χ^2 = 2.05, p = 0.152) or moderate form (χ^2 = 2.05, p = 0.152) in incisors in relation to age groups of children.

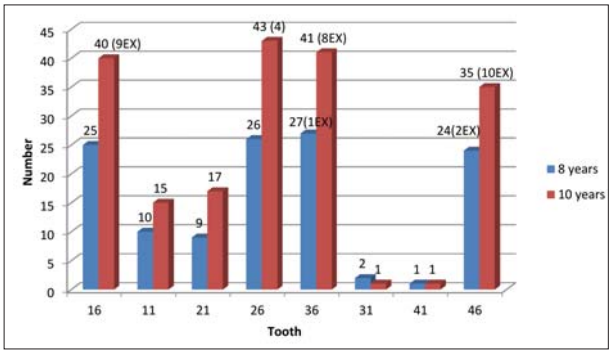

Figure 1. Frequency of hypomineralized changes in molars and incisors per tooth and extracted teeth due to MIH

The mild form in all teeth with MIH changes is statistically more common in children aged eight years (χ^2 = 30.94, p < 0.001), whereas both the severe form (χ^2 = 16.48, p < 0.001) and severe form with extracted teeth (χ^2 = 34.39, p < 0.001) are statistically more common in children aged 10 years. There is no statistically significant difference in the moderate form in all teeth with MIH changes in relation to age groups (χ^2 = 0.03, p = 0.86).

The analysis of data has revealed that the upper right (16) and lower left first permanent molar (36) are most often (21.5%) affected by hypomineralized changes in children aged 10 years. In children aged eight years, lower left first permanent molar (36) is the most common tooth with hypomineralized changes. Of all the incisors in both jaws, upper right and upper left central incisors are most often affected by MIH in both age groups of children. Central lower incisors are slightly affected by the mentioned

changes. Lateral incisors in the upper and lower jaw, in the examined children, are not affected by the changes. All the teeth extracted due to hypomineralization are molars. The results have shown that the lower right first permanent molar (46) is the most common extracted tooth, which is 2.9% of teeth affected by MIH. The upper right (2.6%) and lower left first permanent molar (2.2%) are also commonly extracted teeth due to MIH, in children aged 10 years, which represents a statistically significant difference (Figure 1).

DISCUSSION

Analyzing and determining the frequency, prevalence and severity of MIH is not easy. Particularly difficult is the inevitable subjectivity in assessing the severity of the changes, and also non-standardized criteria used in various studies to diagnose the disease. However, the current findings of the prevalence of MIH in the northern part of Kosovo and Metohija are comparable to those from international studies conducted in the same age groups, which indicates that MIH is a significant problem [14, 18, 19, 20].

Prevalence of hypomineralized defects was first tested in this area and found in 12.2% of the total children examined. The same percentage of prevalence of MIH, as in our study, was found in Bosnia and Herzegovina (12.3%) in children aged 12 years [21], while in children aged eight years, in the municipality of Foča in 2008, MIH was recorded in 12.8% of the examined children [19]. Approximate percentage of MIH was found in the study conducted in Italy (Lissone) in 2005 with eight-year-old children, and was 13.7% [22].The relatively high prevalence of MIH (17.85%) was found in children aged six and 14 in Spain [20]. In 2012 in Turkey, in a study conducted with children aged between eight and 11 years, the distribution of MIH was lower (7.7%) [23]. The study done in Libya in 2006 with children aged seven to nine years, showed significantly lower percentage of MIH (2.9%) [24]. Also, the study of prevalence of MIH in the permanent dentition in Chinese children aged 12 years in Hong Kong showed a low prevalence percentage of 2.8% [12]. Analysis of previous epidemiological studies confirms that the percentage of MIH prevalence is not negligible. Large differences in the results on the prevalence of HIM in different countries can be explained by different age groups of the respondents, local environmental factors, and different criteria used in the studies [13, 18, 20, 25].

The selected children were aged eight years, because in that age group all four first permanent molars and incisors were present, prevalence of dental caries was still low, so there was less chance that carious lesions mask hypomineralization. This study also included children aged 10 years to examine the progression of changes in molars, which largely depend on the post-eruptive tooth age or patient age. By analyzing the results of the hypomineralized changes in this study, it was found that there was no statistically significant difference in the occurrence of these changes in respondents aged eight and 10 years. Contrary to this study, Sönmez et al. [23] found that MIH was more common in children aged eight compared to children aged 11 years (8.5% versus 6.5%).

Some studies speculated about the possible significance of sex in the development of MIH. Some studies point to a slightly higher prevalence of MIH in male respondents [6]. Neither this nor the studies in Libya nor in Bosnia and Hercegovina, nor other studies that addressed these issues, confirmed sex predisposition [19, 21, 23–26].

Review of literature shows that there is little data about individual criteria for MIH. The prevalence of certain criteria is difficult to compare. Despite the criteria being clearly determined, the details of the criteria are not clearly defined. One of the first concerns is the size of the lesion. In our research, we included only defects (opacities) larger than 1 mm in diameter. The same criterion, considering the size of the lesion, was used by Jälevik et al. [27] to determine the prevalence of MIH. Some studies included only defects larger than 2 mm [21, 22, 28].

By analyzing individual criteria for MIH in this study, it was found that demarcated opacity is the most common criterion, which is in agreement with other studies [19, 23, 25]. Post-eruptive enamel breakdown was found in 25.3% of the teeth and it was more common in molars compared to incisors. This finding is in agreement with other studies because the changes in incisors are not as extensive as in the first permanent molars and rarely, except for the color change, result in post-eruptive enamel breakdown, primarily because of no pressure during mastication [18, 25]. An atypical restoration, in our study, was found in 22.2% of the examined teeth and only in molars. The same percentage of teeth with an atypical restoration was found in the respondents in Bosnia and Herzegovina [21]. In the study in Lithuania, atypical restoration was found in 16.9% of the respondents with MIH [26].

In the present study, all teeth extracted due to hypomineralized changes or their complications were molars. In the study in the municipality of Foča conducted in 2008, tooth extraction due to MIH was found in 5.6% of children, i.e. 19% of the total number of teeth with MIH, and they were all molars [19]. In a study from Lithuania there were no teeth extracted due to MIH [26].

Un-erupted teeth due to hypomineralized changes were not found in this study. These data are in agreement with the majority of studies that dealt with MIH [19, 22, 23, 29].

By analyzing individual criteria for MIH, in relation to age, it was found that both atypical restoration and tooth extraction due to MIH are statistically more common in children aged 10 years. This finding points to the necessity of early diagnosis of MIH, which will enable timely prevention and treatment of this condition.

The mild form of hypomineralized changes in this study is the most common in all teeth with MIH. Compared to all MIH studies, we confirmed that the mild form prevailed [19, 23]. The severe form of hypomineralization was found in 5.8% of children, i.e. 27.8% of teeth, of which all were molars. The severe form was not found in incisors. In a study in conducted in Sweden in 2001 a similar percentage was recorded: severe form was found

in 6.3% of the respondents [27], while significantly lower percentage was found in a group of children who lived in the Italian city Lissone (0.4%) [22]. By analyzing the presence of some degree of severity in relation to age, it was found that the severe form was more common in children aged 10 years, while the mild form was more common in younger children. There were no significant differences in the mean values of moderate forms. These results are in agreement with findings of other authors [18, 23, 25, 30]. Frequent prevalence of the severe form in older children can be explained by the fact that hypomineralized molars, depending on the degree of hypomineralization, are brittle, fragile, and as such prone to post-eruptive enamel breakdown under the influence of chewing forces. Also, due to hypersensitivity to temperature stimuli, children with these changes can have painful sensations when brushing teeth. This is the reason why they avoid regular oral hygiene This leads to accumulation of dental plaque, together with rapid progression of caries lesions that result in crown destruction and tooth loss.

This study confirms the findings of other studies, where the first permanent molars are most often affected by MIH changes, while the lateral incisors are least affected [12, 18, 21, 29]. When analyzing the presence of MIH in each tooth, it can be concluded that the upper right (16) and lower left first permanent molar (36) are most often affected by hypomineralized changes. Data from the literature suggest that the changes are more common in maxillary incisors, which is also confirmed in our study [19, 23, 27, 29].

CONCLUSION

Hypomineralization of the first permanent molars and incisors is very common in children in many European countries, as well as in our country, which was confirmed by our study. In this study, no significant difference in frequency of MIH changes was found in respondents in relation to age and sex. In relation to the degree of severity of MIH changes, it was found that the severe form was more common in children aged 10 years, while the mild form was more common in younger children. This finding indicates that MIH is inadequately treated and not recognized on time due to the rapid post-eruptive enamel loss, hypersensitivity of the affected teeth, rapid and early caries progression (especially in the first permanent molars), and dental wear. Therefore, monitoring teeth eruption and making an accurate and timely diagnosis of MIH would significantly contribute to better prevention and treatment of these developmental defects.

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Учесталост, карактеристике и степен изражености хипоминерализације на првим сталним кутњацима и секутићима код деце која живе на подручју северног дела Косова и Метохије

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САЖЕТАК

Увод/Циљ Хипоминерализација кутњака и секутића (ХКС) релативно је честа развојна аномалија која се карактерише појавом хипоминерализованих дефеката глеђи на првим сталним кутњацима и секутићима.

Циљ овог рада био је да се испита учесталост хипоминерализације на првим сталним кутњацима и секутићима код деце узраста осам и десет година на подручју северног дела Косова и Метохије.

Методе У истраживању је било укључено 712 испитаника – 289 узраста осам година (40,6%) и 423 (59,4%) узраста десет година. За дијагнозу обољења примењени су критеријуми по *Weerhejim*-у и одређен је степен израженості промена.

Резултати Учесталост хипоминерализованих промена на првим сталним кутњацима и секутићима код испитиване деце на овом подручју износио је 12,2%. Код деце од осам година учесталост ових промена је мања (10,7%) у односу на испитанике од десет година (13,2%). Ограничена замућеност глеђи била је учесталија код деце млађег узраста, док је код деце старијег узраста учесталија атипична рестаурација и екстракција зуба као последица хипоминерализације. Блага форма је учесталија код деце од осам година, док је код деце од десет година учесталија тешка форма, као и тешка форма која укључује и екстрахиране зубе. Резултати показују да су први стални кутњаци најчешће захваћени хипоминерализованим промена.

Закључак Проценат од 12,2% испитаника са променама ХКС на подручју северног дела Косова и Метохије није занемарљив. Овакво стање указује на неопходност ране дијагностике да би се благовременом превенцијом и терапијом спречиле и ублажиле компликације овог обољења.

Кључне речи: хипоминерализација; кутњаци, секутићи; деца; учесталост

DOI: https://doi.org/10.2298/SARH160719063O
UDC: 615.46

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Histological evaluation of tissue reactions to newly synthetized calcium silicate- and hydroxyapatite-based bioactive materials – *In vivo* study

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SUMMARY

Introduction/Objective Development of materials which could be used as biological bone substitutes is one of the most valuable and active fields of biomaterial research.
The goal of the study was to research the reaction of tissue on calcium silicate- (CS) and hydroxyapatite-based (CS-HA) newly synthesized nanomaterials, after being implanted into the subcutaneous tissue of a rats and direct pulp capping of rabbit teeth.

Methods The tested materials were implanted in 40 Wistar male rats, sacrificed after seven, 15, 30, and 60 days. The direct pulp capping was performed on the teeth of rabbits. Cavities were prepared on the vestibular surface of the incisors. The animals were sacrificed after 10 and 15 days. The control material was mineral trioxide aggregate (MTA). Histological analysis covered the tracking of inflammatory reaction cellular components, presence of gigantic cells, and necrosis of the tissue.

Results Seven days after the implantation, the strongest inflammatory response was given by the MTA (3.3 ± 0.48), while CS and CS-HA scored 3 ± 0.71. After 60 days, the rate of inflammatory reactions dropped, which was the least visible with CS-HA (0.2 ± 0.45). The least visible inflammatory reaction of the rabbits' pulp tissue was spotted with the CS (1.83 ± 0.75), than with the MTA and CS-HA (2.67 ± 1.53, 3 ± 0.63).

Conclusion The newly synthesized materials caused a slight reaction of the subcutaneous tissue. CS-HA showed the best tissue tolerance. Nanostructural biomaterials caused a slight to moderate inflammatory reaction of the rabbits' pulp tissue only in the immediate vicinity of the implanted material.

Keywords: biocompatibility; calcium silicate system; hydroxyapatite; mineral trioxide aggregate

INTRODUCTION

Biocompatibility of dental materials and the constraints imposed by their toxicity in contact with dental and other oral tissues are an important segment in the research of the newly synthetized materials. Cytotoxicity of the material can cause inflammatory reaction in contact with the surrounding tissue, significantly affecting the therapy outcomes [1, 2]. Therefore, multiple testing of synthetized materials is required in order to ensure their reliable application in day-to-day clinical practice. For many years now, the scientific community has been focusing on the need to evaluate the biological properties of new materials at the pre-clinical stage by using various in vitro and in vivo methods, as these mostly remain in long-term contact with local cells and tissues [3]. In this interaction, the onset of the foreign body reaction usually takes place immediately after the material is implanted (going through the stages of inflammation and healing, and involving a number of different cell types), making the in vivo biocompatibility testing one of the most important steps towards their prospective clinical application.

While the evaluation made based on in vitro assays may be faster in rendering biological interaction data, the reliability of such data remains questionable compared to the data obtained in more complex in vivo conditions. In vivo assays are normally carried out on animal models (implantation in subcutaneous, muscle, bone, or other tissues), and they precede assays on target animal tissues and human clinical trials [4].

Subcutaneous tissues are tissues of choice for the biocompatibility evaluation of the implanted material. In the opinion of the authors they are found on highly accessible sites, enabling the evaluation of the biological reactions to biomaterials or, in other words, facilitating detection of inflammatory tissue reactions to the agents in the implanted material [3]. Histological examination is the most frequently used method in the research of tissue compatibility and its capability to restrict the inflammatory reaction to the implanted material [5–9].

The direct pulp capping is a therapeutic procedure for preserving the dental pulp vitality by covering the exposed pulp injury with materials that will foster reparative dentine formation [10]. The direct capping material plays a key role in the course of this treatment as it comes into direct contact with the pulp tissue. Although calcium hydroxide has been a direct pulp capping

Received • Примљено: July 19, 2016
Accepted • Прихваћено: October 4, 2016
Online first: March 10, 2017

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agent in most frequent and longest use [10, 11, 12], the practice of many years has also shown frequent unforeseeable outcomes of this therapy [13, 14]. In the era of regenerative endodontics, new procedures and materials for biological therapy and tooth revitalization have been introduced, including biomaterials such as calcium phosphate, calcium silicate, and bioactive glass-ceramic cements. These bioactive dental materials are essential for a better and more effective regenerative endodontic treatment [15].

The objective of this study was to investigate the tissue inflammatory response to newly synthetized nanomaterials based on calcium silicates (CS) and hydroxyapatite (CS-HA) in in vivo conditions by a) implanting the material into the subcutaneous tissue of rats, and b) direct capping of exposed dental pulp of rabbits.

METHODS

Permission for the experimental work on animals was obtained from the Ethical Committee of the Belgrade University Faculty of Dental Medicine (No. 36/5, April 4, 2012). The experiment was conducted in line with the international standards ISO 7405 and ISO 10993-2 (animal welfare requirements) [16, 17]. The initial step in carrying out this study was an innovative method of bio-ceramic material synthesis (with nanoscale particles) applied for the first time by V. Jokanović. The materials used in this study were nanostructured CS with and without the addition of 40% HA-CS mixed with distilled water in the 2:1 ratio of powder to water, according to the recommended protocol. The control material was mineral trioxide aggregate (ProRoot MTA, Dentsply Maillefer, Ballaigues, Switzerland) mixed in a 3:1 ratio, according to the instructions of the manufacturer.

Design of the subcutaneous implantation experiment

Forty male rats (Wistar albino), between 2.5 and 3 months old and weighing on average 350 g each, were used. After the animals were anaesthetized, two-centimeter-long incisions in the animals' backs were made in the head-to-tail direction. Using the blunt dissection to the right and left of the spine, two pockets approximately 15 mm deep were opened and sterile polyethylene tubes with the test materials were implanted using sterile clinical tweezers. Polyethylene tubes 10 mm long and with internal diameter of 1 mm, half-filled with freshly mixed materials (CS, CS-HA, and MTA) were implanted in the subcutaneous tissue. The empty half of the tube was used as the negative control. Each animal received two tube implants. The tube with the test material was inserted on the right side of the spine and the tube with MTA on the left. The tubes were positioned so that the material was at all times oriented towards the head, and the empty half of the tube towards the tail. By random selection the animals were divided into two groups of 20 for each tested material. Ten animals (five of each material) were sacrificed in each of the four observation periods – days 7, 15, 30, and 60.

Tissue samples together with polyethylene tubes were fixed in 10% buffered formalin. Then the polyethylene tubes were removed. The tissue was then prepared for light microscopy in a standard way, involving dehydration in a series of ethanol solutions of increasing concentrations, illumination in xylol, and paraffin embedding. Paraffin samples 4 μm thick were stained in haematoxylin and eosin. Microscopic slides were analyzed in an optical microscope (BX-51, Olympus Corporation, Tokyo, Japan) and microphotographs were taken by a digital camera (CD video camera, PixeLink, Gloucester, Ontario, Canada) connected to a 19" PC screen (Dell Inc., Round Rock, TX, USA) [2].

In line with international standards (ISO 10993-6, Biological evaluation of medical devices – Part 6: Test for local effect after implantation), local tissue reactions where the materials had been implanted were evaluated. In the histological examination of the prepared samples, the parameters were analyzed qualitatively and semi-quantitatively (modified according to Scarparo et al. [6] and Lotfi et al. [18]): a) inflammatory response (0 – no inflammation; 1 – minimal (< 25 inflammatory cells); 2 – mild (26–50 inflammatory cells); 3 – moderate (51–100 inflammatory cells); 4 – severe (> 100 inflammatory cells); b) vascular congestion (0 – absent; 1 – minimal; 2 – mild; 3 – moderate; 4 – severe, involving blood vessel burst).

Design of the direct pulp capping experiment

The animal model used in this experimental part of the study were four rabbits (Oryctolagus cuniculus) of both sexes, from different broods, aged about 12 months, average weight of 4 kg, on controlled diet and receiving daily care. For the purposes of the surgical procedure, the general dissociative anesthesia (xylazine, ketamine, acepromazine) was administered. The average duration of anesthesia was 100 minutes.

The surgical procedure was carried out in aseptic conditions and so as to ensure minimum trauma. Each tooth was cleaned, dried and disinfected (30% hydrogen peroxide and 5% iodine tincture). Class V cavities were then created in the gingival third of vestibular surfaces of incisors by using round, water-cooled diamond burs. A new set of diamond and carbide burs was used for each animal. In the middle of the cavity, pulp was exposed using sterile, round bur. Cavities were gently dried, with no pressure exerted, using sterile cotton wool balls. Freshly mixed material was applied to the exposed pulp. All cavities were closed with glass ionomer cement (GC FUJI VIII, GC Corporation, Tokyo, Japan) as a definitive filling. The material used for direct capping of the exposed pulp was MTA and it was implanted in the upper right maxillary incisor, while the other three incisors were implanted with CS and CS-HA, in two rabbits respectively. The animals were sacrificed after 10 and 15 days, by intravenous injections. Having removed soft tissues, the teeth in the alveoli were cut with a diamond disc. The samples were fixed in 10% formalin and decalcified. Following the decalcification, the tissue was fixed in semi-enclosed benchtop tissue processor (Leica TP1020, Leica Biosystems, Wetzlar, Germany) and

then embedded in paraffin blocks. Serial tissue sections 5 μm thick (eight from each sample) were cut from the paraffin blocks. The slides were stained in haematoxylin and eosin, following the standard procedure.

The microscopic slides were examined by optical microscopy, using Olympus Cell-B software package and Olympus 5 microscope at magnifications of ×10, ×40, ×100, and ×200. In addition to the software, the pathohistological parameters were assessed qualitatively, semi-quantitatively, and quantitatively. Examination of every tooth included the scoring of the following parameters (scoring system 1–4): a) pulp inflammatory response: i) intensity (1 – no inflammation, 2 – mild, 3 – moderate, 4 – severe, > 25 inflammatory cells), ii) extent of inflammation (1 – no inflammation, 2 – mild, inflammatory cells close to the exposed portion of the pulp, 3 – moderate, inflammatory cells in the area of coronal pulp, 4 – severe, whole coronal pulp is infiltrated or necrotic), iii) general state of the pulp (1 – no inflammation, 2 – with inflammation, 3 – abscess, 4 – necrosis); b) other findings in the pulp (gigantic cells, direct capping material particles, presence of microorganisms).

In the statistical analysis, the non-parametric Kruskal–Wallis test with Dunn's post hoc test for inter-group comparisons was used. The statistical analysis was made using the Minitab 16 software package (Minitab Inc., State College, PA, USA).

RESULTS

The results of the histological examination of subcutaneous implantation are shown in Table 1 and in Figures 1–6.

In the examined samples seven days after the implantation of CS and CS-HA, a moderate inflammatory reaction was observed (score 3) (Figure 1), while the connective tissue with the MTA implant showed somewhat more intensive inflammatory reaction (3.3) with diffuse and focal subcapsular and perivascular inflammatory infiltrates (Figure 2). Inflammatory infiltrate cells included lymphocytes and plasmocytes, while rare granulocytes were observed in only two samples. The connective tissue had a small number of normal-structure blood vessels with signs of moderate congestion, and it could receive scores 2.8 and 2.5 for CS and CS-HA, respectively, and score 2.4 for MTA.

In the observed slides 15 days after implantation of CS and CS-HA materials, mild inflammation was observed (scores 2 and 1.6, respectively) (Figure 3), while the MTA group displayed mild to moderate inflammation (score 2.3). Blood vessels were of the normal number and with signs of minimal venous stasis, receiving score of 0.8 in the CS-HA group, while the MTA and CS groups displayed minimal and mild congestion, respectively (scores 1.3 and 1.6, respectively).

In the observed samples 30 days after the implantation, the connective tissue was of normal structure and with minimal number of inflammatory cells in the CS and CS-HA groups (scores 1.6 and 1.4, respectively) (Figure 4). In the MTA group, the surrounding tissue in most samples showed signs of mild inflammation (score 1.9).

Table 1. Mean value of inflammation scores and standard deviation

Material	7 days	15 days	30 days	60 days
CS	3 ± 0.71	2 ± 0.71	1.60 ± 0.55	0.5 ± 0.58
CS-HA	3 ± 0.82	1.6 ± 0.55	1.40 ± 0.55	0.2 ± 0.45
MTA	3.3 ± 0.48	2.3 ± 1.06	1.90 ± 0.88	0.44 ± 0.73
Control	2.5 ± 1.43	1.9 ± 0.74	1.50 ± 0.53	0.67 ± 1

CS – calcium silicates; CS-HA – calcium silicate with hydroxyapatite; MTA – mineral trioxide aggregate

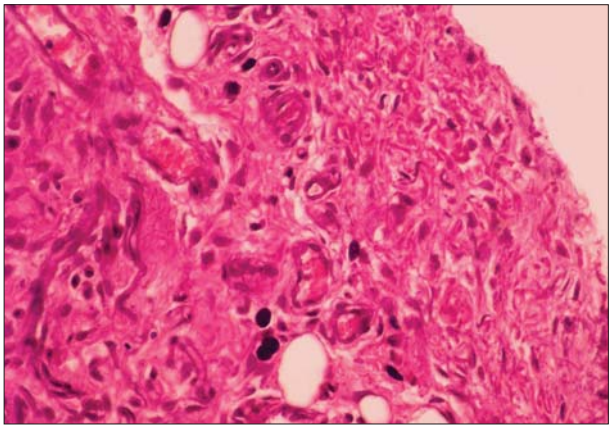

Figure 1. Calcium silicate implantation after seven days; mild inflammatory reaction is visible, while rare monocytes, lymphocytes, and granulocytes can be found in infiltrate; blood vessels with signs of moderate congestion (H&E, ×40)

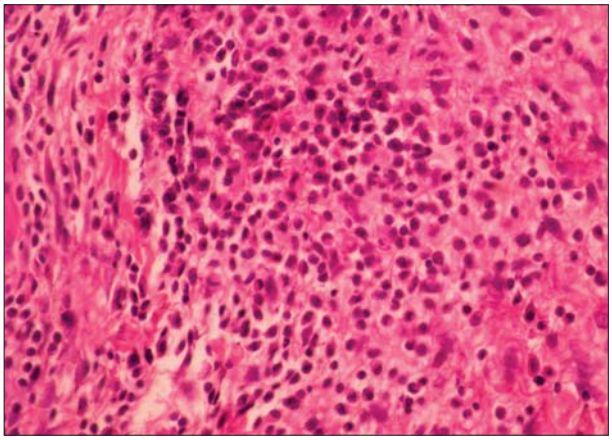

Figure 2. Mineral trioxide aggregate implantation after seven days; visible focal and diffuse inflammatory reaction of connective tissue (H&E, ×40)

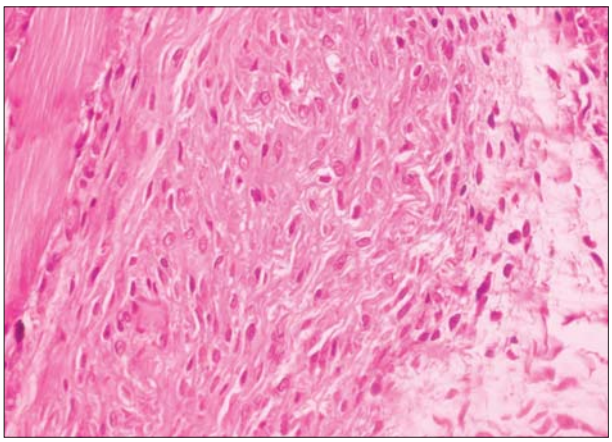

Figure 3. Calcium silicate implantation after 15 days; connective tissue of mostly preserved integrity is visible, no venous stasis (score 1.4); presence of lymphocytes and plasmacytes confirms chronically inflammatory reaction (H&E, ×40)

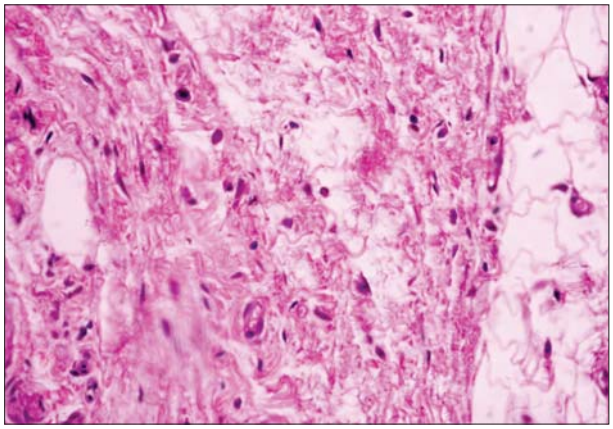

Figure 4. Calcium silicate implantation after 30 days; integrity of the connective tissue is visible, with minimal number of inflammatory infiltrate cells (score 0.6) (H&E, ×40)

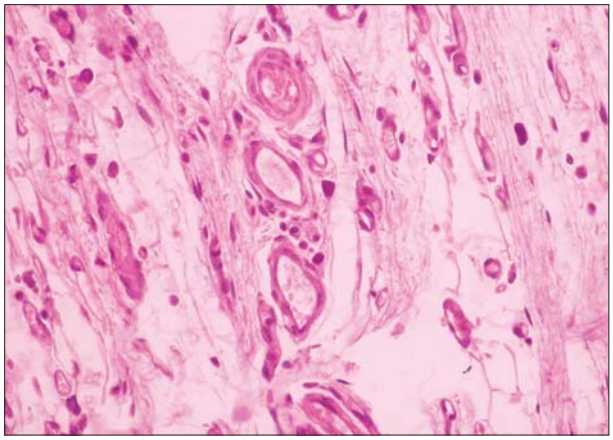

Figure 5. Calcium silicate implantation after 60 days; loose connective tissue with preserved integrity is visible; single cells of inflammatory infiltrate are present (score 0.5) as well as an increased number of new blood vessels, which indicates tissue remodeling (H&E, ×40)

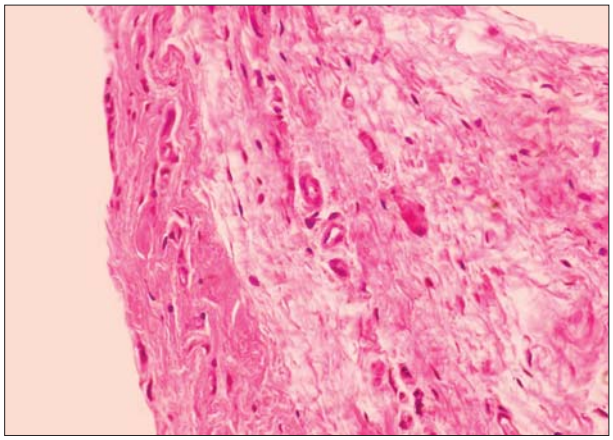

Figure 6. Hydroxyapatite-based implantation after 60 days; subcapsular connective tissue with preserved integrity (score 0) is visible (H&E, ×40)

The connective tissue was with a usual number of blood vessels and no signs of venous congestion, receiving score 0.6 in the CS and CS-HA groups, and score 0.8 in the MTA group.

In the tested samples, 60 days after the beginning of the experiment, loose connective tissue with individual cells of inflammatory infiltrate could be observed [score

Table 2. Mean inflammation score values of the tested materials

Material	Intensity	Extent	General state of the pulp
CS	1.83 ± 0.75	2.17 ± 0.75	1.5 ± 0.55
CS-HA	3 ± 0.63	2.17 ± 0.41	2.17 ± 0.41
MTA	2.67 ± 1.53	2.67 ± 1.15	2.67 ± 1.15

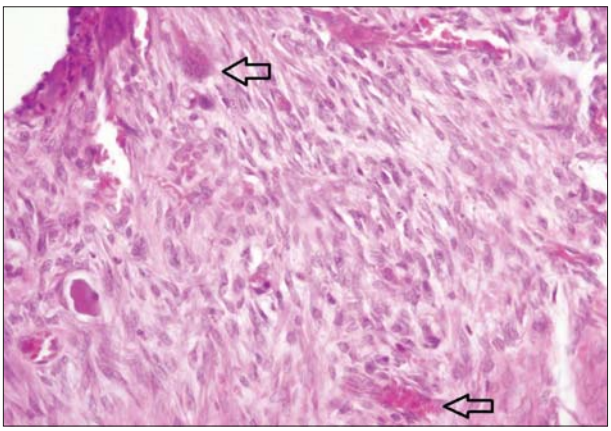

Figure 7. Direct pulp capping with calcium silicate; reactive chronical inflammation with rare mononuclear cells and focally present giant type cells around the foreign body (arrows) (H&E, ×400)

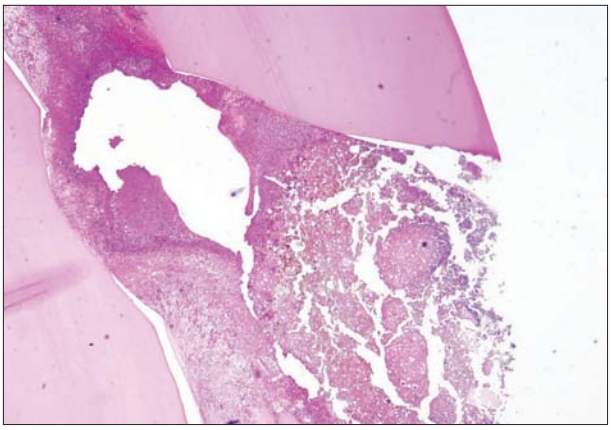

Figure 8. Direct pulp capping with hydroxyapatite; tooth enamel cavity; implanted material surrounded with mild to intensive pulp inflammatory reaction with focal necrosis (H&E, ×40)

0.4 for CS-HA and MTA) (Figure 6) and score 0.5 for CS (Figure 5)]. The blood vessels of normal structure and in a normal number, with no signs of venous stasis observed in any of the tested material (0).

The histological examination results of the rabbit teeth direct pulp capping are shown in Table 2 and Figures 7–11.

The pulp tissue below the CS material showed signs of mild inflammatory reaction (Figure 7). The general state of the pulp suggested a mild inflammatory reaction (score 2). While the difference between the tested materials (CS and CS-HA) was not statistically significant, it was significant between CS and MTA ($p = 0.040$).

All the observed samples where CS-HA was used as the direct capping material showed signs of moderate inflammation (Figure 8). The general state of the pulp inflammation received a score of 2. A significant number of gigantic cells and scattered particles of the material were observed in the samples (Figure 9), which was statistically

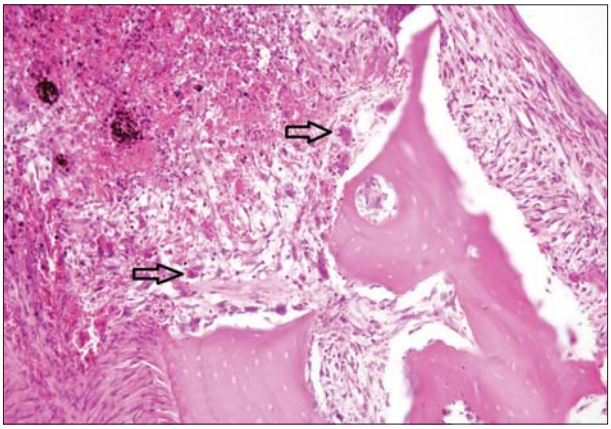

Figure 9. Loose particles of hydroxyapatite visible in pulp tissue surrounded with giant type cells around the foreign body (arrows) (H&E, ×200)

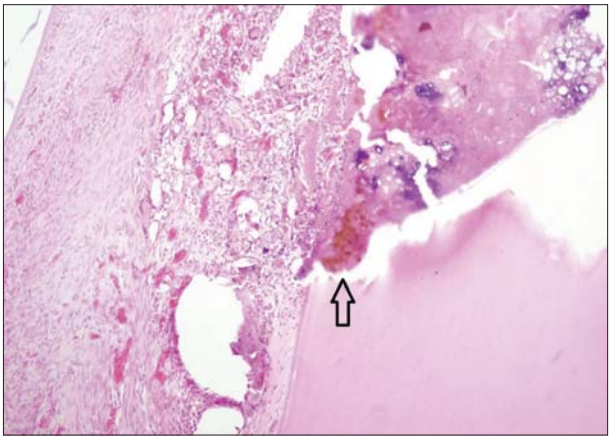

Figure 10. Direct pulp capping with mineral trioxide aggregate; necrotic zones spread around the mineral trioxide aggregate particles; blood vessels show signs of acute hyperemia (H&E, ×100)

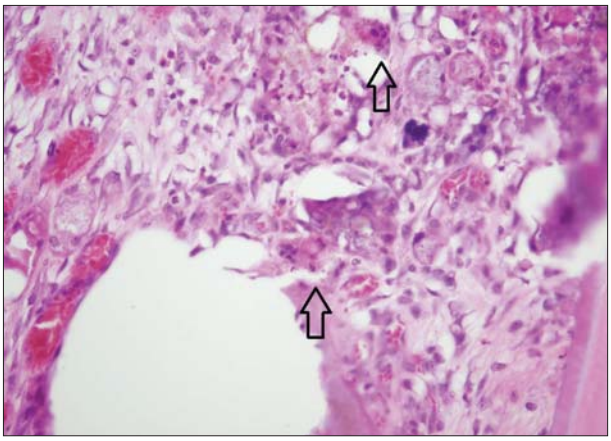

Figure 11. Direct pulp capping with mineral trioxide aggregate; mild inflammatory reaction with phagocyted material, a number of foamy histiocytes and giant type cells around the foreign body (arrows) (H&E, ×400)

significant in the CS ($p = 0.001$) and MTA groups ($p = 0.033$). No bacteria were detected in any of the samples.

All the samples with MTA as the direct capping material showed signs of mild to moderate inflammation, with a few inflammatory cells immediately around the site of the exposed pulp. An intensive inflammatory reaction was detected in one sample, where inflammatory cells infiltration throughout the coronal pulp and necrosis were present (Figure 10). The presence of a small number of gigantic cells was detected in two samples; the particles of the phagocytized material were also observed (Figure 11). No bacteria were detected in any of the samples.

DISCUSSION

At present, enormous progress has been achieved in synthesizing a number of new materials used in clinical practice.

The materials tested in this study were newly synthesized nanomaterials based on tricalcium and dicalcium silicates and hydroxyapatites (CS and CS-HA). Their structure and biological properties were compared with those of MTA, which is the golden standard of tricalcium silicate cements.

Understanding the mechanism of interaction between biological fluids or cells and endodontic materials is key to assessing new materials used in diagnostics and therapy, and to avoiding materials' harmful reactions after their use [19]. Nanoparticles of different size and chemical structure typically get deposited in mitochondria, causing considerable structural damage due to the reactive oxygen species synthesis that leads to oxidative stress. Mitochondria are the main locations of reactive oxygen species production in a cell, which can result in the generation of a hydroxyl radical – one of the most potent oxidizing agents in nature. Oxidative stress is the most common cause of cell injury.

Nanostructured calcium silicates, CS and CS-HA (with particles in the range of 117–477 nm), in the initial in vitro assays designed to assess genotoxicity and cytotoxicity showed the absence of harmful cell effects within the parameters of the Comet and the MTT assays [20, 21].

In vitro studies are fundamentally different from in vivo ones where proteins, tissue fluids, and other factors can reduce the toxic effects of the material [22]. In vivo assays render more comprehensive and clinically more relevant information about the tissue response over a protracted time period. The soft tissues' histological reaction to biomaterial has been a long established and frequently used method of biocompatibility assessment [7, 9]. These assays are highly reliable in evaluating the tissue irritation, and the interaction of tissue and biomaterial. Tissue reaction to an implant is a cumulative pathophysiological consequence of a) the healing of an acute injury sustained due to a surgical wound and the presence of implant, b) possible chronic inflammation, c) the surrounding tissue repair while adapting to the implant.

This experiment monitored short-term, but also long-term, effects of the materials on the tissue, since these data are relevant to the clinical use of the endodontic materials.

Monitoring of a specific response to a foreign body following the material implantation starts with *inflammation,* continuing through the stages of *wound healing* with the involvement of various cell types being specific indicators of the tissue repair stage. With the inflammatory process attenuating, the total number of inflammatory cells decreases while the wound healing process moves towards formation of granulation tissue and *fibrous encapsulation* of the implanted material.

The tissue surrounding the tested bioceramic materials (CS, CS-HA) showed the highest level of inflammation in the first 15 days, with moderate disruption in the connective tissue structures. The connective tissue around the MTA showed signs of the most severe inflammatory reaction with diffuse and focal subcapsular and perivascular infiltrates, which was evaluated as moderate and severe inflammation. Other researchers also reported such a powerful tissue response, observing even coagulative necrosis and dystrophic calcifications [7]. A number of factors initially caused such a severe inflammatory reaction to MTA. High pH values of the freshly mixed material, heat release upon setting, and stimulation of inflammatory cytokines (interleukin 1 and interleukin 6) contribute to such a powerful tissue response to MTA [18, 23, 24].

Tissue reaction to empty tubes (negative control) in this experiment is similar to findings reported by other researchers [9, 22, 24]. It was most severe on the seventh and the 15th day, with cellular infiltrate dominated by lymphocytes and plasmocytes, which is indicative of a chronic inflammatory reaction. The gigantic cells detected in some sites suggest the tissue's reaction to a foreign body. This reaction could partly be a result of surgical trauma sustained upon implanting the tubes in the tissue. At the end of weeks four and eight, the inflammatory infiltrate cells were disappearing, and the tissue at the point of contact with the tube was encapsulated. This suggests the body's capacity to contain the inflammatory reaction thus preventing further tissue damage, as confirmed in papers by Sumer et al. [5] and Scarparo et al. [6].

Further into the tissue repair process (after 30 and 60 days), significant decrease in the inflammation intensity, disappearance of inflammatory infiltrate cells, but also tissue repair and remodeling were observed in all tested materials. The inflammatory response after four and eight weeks of the experiment can be explained by inducing the release of proinflammatory cytokines by released particles of the hydroxyapatite layer formed on the surface of the bioceramic materials. This also suggests good interaction between the material and the cells from the surrounding tissue, which is a sign of good biocompatibility of the materials.

The pulp tissue response to the implanted material usually starts with an acute inflammation, which needs not be present in all cases [25]. The topography and chemistry of the surface of newly synthetized materials plays an important role in odontoblast adhesion to biomaterial. It is a known fact that micro- and nanotopography of the surface and adsorbed proteins have direct influence on the cell behavior and activity, primarily with respect to their adhesion and retention at the point of application [26].

Nanostructured CS cements show increased osteoblast adhesion, proliferation, and differentiation, since the bone itself has nanostructure, and the crystal size and geometry can modify the response of the surrounding tissue. The interaction between the direct capping material and the injured pulp tissue, and the ways in which the healing and repair processes are initiated and developed are still not fully clear. While many hypotheses exist, recent studies have accorded the main role to growth factors in angiogenesis, mobilization of progenitor cells, differentiation, and, finally, biomaterial-assisted mineralization [27].

As a consequence of experimental perforation, but also of the initial effect of the tested materials, a mild to moderate inflammatory reaction was detected in all observed tooth samples. The inflammatory infiltrate was in close proximity of the implanted material and was not spreading further into the coronal pulp. The weakest inflammatory reaction, sporadically with total absence of inflammation, was observed in samples where CS cement was implanted as the direct capping material, which agrees with the findings of other researchers, who find up to 50% samples with no signs of inflammation in the early stage [12, 28]. Moderate inflammation was detected in the CS-HA and MTA samples, which is confirmed by similar experiments where inflammation appeared in more than 62% of the MTA samples after two weeks, with the intensity declining after eight weeks. This initially severe inflammatory response is a result of the pulp tissue coagulative necrosis in contact with MTA (pH being 9–10). This zone has a stimulating effect on the surrounding vital pulp tissue, which initiates a string of healing processes. Due to bio-degradation of the material in contact with the tissue fluids, Ca and P ions are released, creating alkaline environment, which has a favorable effect on adhesion and proliferation of cells involved in the healing processes [12, 29]. Asgary et al. [30] observed that new endodontic cements having similar structure as MTA but improved physical and chemical properties – they include the nanomaterials tested – show better pulp response (weaker inflammatory reaction) and thicker dentine bridge then MTA at a later stage.

Biomaterials are normally tested on animals, since they are a model of the environment one can find in humans. Nevertheless, animas are characterized by a huge range of differences with respect to anatomy, biochemistry, physiology, and other variables. In the absence of confirmation from human clinical trials, it is often difficult to draw a conclusion solely on the basis of animal testing. Testing carried out on live systems invariably leads to experimental variability. The more complex the system (human cells versus microorganism cells), the higher statistical variability of testing results can be expected [3].

CONCLUSION

The results shown in the present in vivo study on the animal model have proven that the subcutaneous tissue of rats and the pulp tissue of rabbits have favorable biological response to newly synthetized nanostructured biomaterials (CS and CS-HA). The inflammatory reaction in the subcutaneous tissue was severe only in the initial days after the implantation and its intensity declined as a function of time. In direct pulp capping there was a mild to moderate inflammatory reaction in the close proximity of the implanted material. The tissues showed high tolerance to the implanted materials, which confirms their biocompatibility, as in previous in vitro studies.

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Хистолошке реакције ткива на новосинтетисане биоактивне материјале на бази калцијум-силикатних система и хидроксиапатита – *in vivo* студија

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САЖЕТАК

Увод/Циљ Усавршавање материјала који би могли да се користе као биолошке замене кости једна је од најзначајнијих и најактивнијих области истраживања биоматеријала. Циљ овог рада је био да се испита одговор ткива на новосинтетисане наноматеријале на бази калцијум-силикатних система (КС) и хидроксиапатита (КС-ХА) после имплантације у поткожно ткиво пацова и директног прекривања пулпе зуба кунића.

Методе У поткожно ткиво 40 вистар пацова су имплантирани тестирани материјали, а после 7, 15, 30 и 60 дана животиње су жртвоване. Директно прекривање пулпе је реализовано на зубима кунића. На вестибуларним површинама секутића препарисани су кавитети, а експонирана пулпа је прекривана тестираним материјалима. Животиње су жртвоване после 10 и 15 дана. Контролни материјал у оба експеримента је био минерални триоксидни агрегат (МТА). Хистолошка анализа је обухватила праћење ћелијске компоненте запаљења, присуства гигантских ћелија и некрозе ткива.

Резултати Седам дана после супкутане имплантације најјачи запаљенски одговор дао је МТА (3,30 ± 0,48), док је за КС и КС-ХА он оцењен са 3,00 ± 0,71. После 60 дана дошло је до опадања знакова запаљења, које је било најмање изражено око КС-ХА (0,20 ± 0,45). Најмање изражена запаљенска реакција пулпног ткива кунића уочена је код материјала КС (1,83 ± 0,75), затим код МТА и КС-ХА (2,67 ± 1,53, 3,00 ± 0,63).

Закључак Новосинтетисани материјали су изазвали благу запаљенску реакцију поткожног ткива пацова, а КС-ХА је показао најбољу ткивну толеранцију. Наноструктурни биоматеријали КС и КС-ХА су узроковали благу до умерену запаљенску реакцију пупног ткива кунића само у непосредној близини имплантираног материјала.

Кључне речи: биокомпатибилност; калцијум-силикатни систем; хидроксиапатит; минерал-триоксид агрегат

DOI: https://doi.org/10.2298/SARH161215071V
UDC: 618.19-006-073; 618.19-006-089

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Intraoperative digital specimen radiography in the treatment of nonpalpable breast lesions

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SUMMARY

Introduction/Objective About a third of the breast lesions on mammography are clinically occult. The goals of surgical treatment are to locate, remove, and verify their presence in the removed breast tissue. Standard specimen mammography (SSM) has been an official procedure for the latter, while intraoperative digital specimen radiography (IDSR) was introduced recently.
The aim of this study was to evaluate the use of IDSR versus SSM and possible benefits regarding the duration of the procedures (operating room occupancy), availability of digital mammography for additional number of patients, surgeon productivity, and the quality of performed services.

Methods A retrospective chart review of 109 patients who underwent IDSR for nonpalpable breast lesions was performed between January 2014 and June 2016. We compared the difference in the duration of IDSR versus SSM procedure. We also observed the number of re-excisions and evaluated time-saving in the operating room workflow.

Results The average duration of surgery in the IDSR group of patients was 51 minutes, compared to 64 minutes in the SSM group. Every IDSR procedure saved 13 minutes over the standard SSM. That would allow another 28 procedures in the same time frame, with the same quality of service compared with SSM. In that way we increased productivity by 27.5%. Additional operation/surgery was needed for histologically involved surgical margins in three cases (2.75%).

Conclusion The use of new technology resulted in the rationalization of the operative room workflow and gave better productivity. More savings were obtained through the increase of digital mammography capacity for diagnostics, decrease of anesthesia duration, and better management of human resources. The number of "true" re-excisions, involving additional surgery, remained similar after introducing IDSR.
Keywords: nonpalpable breast tumors; specimen radiography; nonpalpable lesion localization

INTRODUCTION

The treatment of breast tumors has significantly advanced thanks to new technologies and new drugs, which have led to better understanding of the nature of the disease. Intraoperative digital specimen radiograph represents such a technology. It shortened the operative time, increased the productivity and decreased the work costs. A number of scientific papers report on the ways to rationalize the work of operating rooms [1, 2, 3].

Screening for breast cancer reveals an increased rate of nonpalpable changes which require the use of various techniques for their localization, followed by histological verification [4, 5]. The use of percutaneous core biopsy and vacuum-assisted biopsy solve diagnostics for a number of lesions without the need for surgery [6, 7, 8]. However, the malignancies confirmed by these biopsies, intermediary histological findings or suspicious malignancies, not suitable for core biopsies because of their localization, size, or other contraindications, should be managed by open biopsy. Localization of such lesions is often performed by a hook-wire, radiotracer (radioguided occult lesion localization), dye or metallic clips placed at the site of percutaneous biopsy, or it can be done by intraoperative ultrasound localization [9–13]. These markers help the surgeon localize and excise the lesion precisely. Because those lesions are often nonpalpable and/or invisible even in the excised tissue, it is necessary to confirm that they have been excised completely [11, 14, 15]. This can be achieved by standard specimen mammography (SSM) or intraoperative digital specimen radiography (IDSR) [16, 17].

The aim of this study was to evaluate the use of IDSR, which replaced previous SSM in December 2013. We assessed possible benefits regarding the duration of surgery and operating room occupancy, availability of digital mammography for additional number of patients, surgeon productivity, and the quality of performed services. This method allows surgeon and/or radiologist to immediately interpret the specimen in the operating room.

Received • Примљено: December 15, 2016
Revised • Ревизија: February 15, 2017
Accepted • Прихваћено: February 20, 2017
Online first: March 14, 2017

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Table 1. Sequence of actions for standard specimen mammography (SSM) and intraoperative digital specimen radiography (IDSR)

SSM	Duration (min.)	IDSR	Duration (min.)
1. The surgeon excises the localized lesion and marks it for better orientation			
2. Transport of the specimen to the Center for Imaging Diagnostics	6	No action	0
3. The technician takes images of the specimen	5	The assisting scrub nurse takes an image of the specimen in the operating room; the surgeon sees it on the monitor; the radiologist can read the image simultaneously on his monitor; consultation is upon surgeon's decision	1
4. The radiologist reviews the image and communicates the results to the surgeon	3	No action	0
5. "Frozen section" analysis of excised margins			
6. Re-excision of the tumor bed			
7. Identification of the sentinel node and axillary dissection if the sentinel node is positive			
8. The end of the procedure			

METHODS

A retrospective study of patient charts and operative protocols gave us the data for 109 patients from 2014 to June 2016. Around one half of the patients underwent preoperative percutaneous biopsy, while the rest was proven by frozen section during their surgery. In case of histologically confirmed or suspicious lesions for infiltrative carcinoma visible on ultrasonography, lesion localization was performed primarily by a radiotracer, which allows sentinel node identification, or we used wire localization with para-areolar injection of the radiotracer. Otherwise, the sentinel node (SN) would have to be examined in a separate procedure. The third method is the use of ultrasound pre- and intraoperatively for lesion localization. The preoperative part is often done by a radiologist by marking the lesion over the skin; intraoperatively, ultrasound is available to surgeons. After lesion localization, all patients underwent breast conserving surgery with or without SN identification, and axillary dissection for metastases.

The durations of the procedure for the IDSR group were obtained from the operative protocols. Since the SSM procedure duration differs from IDSR only in the time needed for transportation, the radiological technicians needed to obtain a specimen mammogram, and the radiologists to interpret it, we simply calculated and added that time to the results we obtained for the IDSR group (Table 1). These measurements were taken three times by a stopwatch and the average time was six, five, and three minutes. We never considered the possibility of mammogram examination of a patient being in progress, which would prolong every procedure for up to 20 minutes.

We divided all the IDSR patients into subgroups in order to precisely evaluate the time needed for tumor excision in the following way: those who had re-excision, SN identification, or both (re-excision + SN), and those who underwent axillary dissection.

In addition, we estimated the total number of re-excisions indicated by the surgeon and radiologist on the ground of IDSR, or other reasons (visual or palpatory findings in the tumor bed), or by the histopathologist (frozen section analysis of the resected margins).

Table 2. Discrepancies between histological findings on percutaneous biopsy and open surgery

No.	Percutaneous biopsy	Surgical biopsy
1.	fibrosclerosis	Ductal CIS, G2, 3,5 mm
2.	papillary tumor	Papillary CIS, G1, 10 mm
3.	papillary tumor	CIS, G?, 8 mm
4.	DCIS, G3	Ductal microinvasive, G3, 3 mm
5.	CDI, G2, DCIS, G2	DCIS, G2
6.	CDI, G2, DCIS, G2, 4 mm	ADH
7.	DCIS, G2, 11 mm	No tumor
8, 9.	CDI, G2, > 40 mm	No tumor after neoadjuvant treatment

CIS – carcinoma in situ; G – grade of the tumor; CDI – carcinoma ductale invasivum; ADH – atypical ductal hyperplasia

We also estimated the total number of additional surgeries for the group. The productivity was assessed in relation to the predicted time-saving with the use of IDSR.

RESULTS

The localization procedures were pre- and intraoperative ultrasound in 66 (60.5%) cases, "hook-wire" in 29 (26.6%), and radioguided occult lesion localization in 14 (12.8%) cases. Out of 109 lesion localizations, 52 (47.7%) patients underwent preoperative percutaneous biopsy; the remaining 57 (52.3%) lesions were proven by frozen section analysis intraoperatively. Twenty-nine (26.6%) patients were verified to have benign lesions, while 30 (27.5%) had infiltrating carcinoma, 28 (25.7%) in situ, and 22 (20.2%) the combination of the two.

There were discrepancies between the histological findings on "core" biopsy and open surgery in nine (8.25%) cases (Table 2).

The average diameter of the invasive tumor was 12.8 mm (range of 4–30 mm), 13 mm (range of 0.2–35 mm) for in situ tumors, and 12.5 mm for the combination of the two (range of 1.5–30 mm).

The average duration of surgery in the IDSR group of patients was 51 minutes, compared to 64 minutes in the SSM group. The average 51 minutes would be prolonged

Table 3. Number of additional surgeries indicated by involved surgical margins

No.	Primary "frozen section" procedure	Additional (second) surgery	Three or more procedures
1.	Free margin (2 mm) on the frozen section	CDI, G2 on the margin	-
2.	No tumor on the margin	CLI, LCIS on the margin	LCIS on the margin solved by mastectomy
3.	No tumor on the margin	DCIS, G2 on the margin	-

CDI – carcinoma ductale invasivum; CLI – carcinoma lobulare invasivum; LCIS – carcinoma lobulare in situ; DCIS – carcinoma ductale in situ

another 11 minutes if re-excision was needed. In case of SN identification, the average time is 66 minutes, a combination of the two would give 83 minutes, and up to 97 minutes for axillary dissection.

The number of re-excisions during the procedure was 33 (30.3%). Additional surgery was needed for histologically involved surgical margins in three cases (2.75%). In one case after the second surgery and lobular carcinoma in situ on the margin, mastectomy was performed (Table 3).

DISCUSSION

It must be said that in SSM procedure potential errors regarding the identification, orientation, and transportation of the excised specimen are possible; this can lead to misinterpretation of resected margins by the histopathologist. Compression of the specimen during mammography imaging can cause overlapping of the margins, which can result in false positive or negative excised margins. The use of IDSR allows for an immediate evaluation of the specimen, confirmation of the complete removal of targeted tissue, and the assessment of excised margins. Our study confirmed that the procedure was shorter by 13 minutes with IDSR compared to SSM. Similar findings were reported in a study by Kaufman et al. [18]. Other studies do not state similar results [19]. Our results showed that preoperative percutaneous biopsy and histological verification of an occult lesion did not shorten the duration of the procedure significantly. It can be explained by the practice of frozen section analysis of surgical margins in all patients in addition to IDSR.

Every IDSR procedure saved 13 minutes over the standard SSM. That would allow another 28 procedures in the same time frame, with the same quality of service compared with SSM. In that way we increased productivity by 27.5%.

We expect more benefits in the future knowing the impact to the learning curve and engagement of all surgeons in the IDSR procedure.

Different studies report positive resection margins in up to 40% of the patients treated with breast conserving surgery [20, 21]. This leads to additional surgery, adverse effects on cosmetics, psychological distress, and higher costs.

According to other authors, the percentage of re-excisions performed in the course of operation is comparable to ours (30%) [17, 22], or lower [18, 23]. IDSR optimizes the surgical procedure because the surgeon, radiologist, and histopathologist have a real-time information about the positive resection margins [22]. It is now possible to exchange opinions directly with no additional time consumption. We had tree re-operations (2.75%) in our study; McCormick et al. [23] found this percentage to be 12%, and after two-view specimen mammography they reduced this rate to 5% [23]. The future goal is to avoid additional operations and increase productivity as much as possible.

CONCLUSION

The use of new technology resulted in the rationalization of the operative room workflow and gave better productivity. More savings were obtained through the increase of digital mammography capacity for diagnostics, decrease of anesthesia duration, and better management of human resources. The use of this method slightly increased the number of re-excisions during the primary operation. The number of "true" re-excisions, involving additional surgery, remained similar after introducing IDSR.

The use of IDSR in everyday practical work has made possible rapid creation and interpretation of specimen radiograms and making immediate surgical decisions based on these images.

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Интраоперативна дигитална радиографија узорка у третману непалпабилних промена дојки

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САЖЕТАК

Увод/Циљ Трећина мамографским прегледом нађених промена у дојци је непалпабилна. Циљ хируршког лечења је да локализује, уклони и потврди промене у одстрањеном ткиву. Мамографија узорка је била стандардна метода за потврду, а интраоперативна дигитална мамографија је уведена недавно.

Циљ рада је био да се одреди евентуална предност интароперативне дигиталне радиографије узорка (ИДРУ) у односу на стандардну мамографију узорка (СМУ), у трајању операције (заузетости операционе сале), ослобађања капацитета дигиталног мамографа за додатни број прегледа, у продуктивности и квалитету пружене услуге.

Методе Ретроспективно је анализирано 109 картона болесника са ИДРУ од јануара 2014. до јуна 2016. Време трајања операције са ИДРУ је упоређивано са временом трајања операције код СМУ. Посматран је број реексцизија и процењена је уштеда у времену рада операционе сале.

Резултати Просечно време трајања операције је 51 минут у групи ИДРУ у односу на 64 минута код СМУ. Свака процедура ИДРУ је донела уштеду у времену од 13 минута у односу на СМУ. Таква уштеда би дозволила извођење још 28 додатних процедура у односу на СМУ у истом временском оквиру, са истим квалитетом услуге. То значи повећање продуктивности за 27,5%. Друга хируршка интервенција је због позитивних ресекционих ивица била потребна у три случаја (2,75%).

Закључак Увођењем нове технологије постигнута је рационализација у раду операционе сале и повећана је продуктивност. Неоспорна је и уштеда због ослобађања капацитета дигиталног мамографа за дијагностичке потребе. Скраћење времена трајања анестезије доноси материјалне уштеде и рационализацију у управљању. Број реексцизија у другом акту је практично непромењен након увођења ИДРУ.

Кључне речи: тумори дојке; непалпабилни; локализација; радиографија узорка

DOI: https://doi.org/10.2298/SARH160811066Q
UDC: 616-002-074:577.1; 616.155.392-085.277.06

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Significance of procalcitonin in bacterial infections among acute leukemia patients with post-chemotherapy agranulocytosis

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SUMMARY

Introduction/Objective Bacterial infection caused by the lack of granulocytes that results from the chemotherapy of acute leukemia is the leading cause of death. At present, there are few sensitive markers to reflect the bacterial infection, and there is no obvious specificity for the diagnosis of infection. Procalcitonin (PCT) is a precursor of calcitonin, and it has been found that PCT is a rapid and accurate marker of infectious diseases in various studies, but its clinical value remained unclear.

This study aimed to explore the clinical significance of PCT levels in patients with acute leukemia who have acquired bacterial infections during the agranulocytosis period post-chemotherapy.

Methods Serum PCT levels were analyzed from samples collected from 92 patients with acute leukemia who had acquired bacterial infections during the agranulocytosis period post-chemotherapy.

Results Serum PCT levels in patients with positive blood cultures were significantly higher than those in patients with negative blood cultures ($p < 0.05$). Gram-negative bacterial infection group was significantly more frequent cause of infection than the Gram-positive group ($p < 0.05$). Furthermore, for patients with positive blood cultures, serum PCT levels were significantly higher in patients who subsequently died than in those who survived ($p < 0.05$).

Conclusion In the period of agranulocytosis combined with bacterial infection that occurred after the chemotherapy of acute leukemia, PCT can show the status of bacterial infection, infected bacterial types and severities.

Keywords: procalcitonin; acute leukemia; bacterial Infections; agranulocytosis

INTRODUCTION

Acute leukemia is a malignant, life-threatening, clonal disease of the hematopoietic tissue. The preferred treatment for acute leukemia is chemotherapy [1]. Due to the characteristics of the disease, and the side effects of chemotherapy drugs, chemotherapy can often result in severe bone marrow suppression, leading to agranulocytosis, thrombocytopenia, and anemia. This can consequently result in increased risk of infection, bleeding, or, in some severe cases, death. With developments in medical technology, administration of hemostatics and transfusion of red blood cells and platelets, the incidence of anemia and death has been greatly reduced.

However, chemotherapy-induced agranulocytosis is still very serious, and can cause sepsis, leading to fever, chills, necrosis, organ failure, or even death [2]. Bacterial infections still contribute to the high mortality rate seen in patients with acute leukemia, in spite of the application of broad-spectrum antibiotics. During the agranulocytosis period, patients with acute leukemia are susceptible to infection with both Gram-positive and Gram-negative bacteria [3].

Culture of pathogens is still the gold standard for their identification in a variety of specimen types. However, there are inevitable limitations to this method, such as the delay in obtaining results [4]. For patients with acute leukemia in the agranulocytosis period post-chemotherapy, time waiting for pathogen culture results is limited, as the onset of infection is acute and severe. To date, there are no specific and sensitive evaluation indexes to monitor the onset of infection and its severity. Thus, the development of methods that enable early diagnosis of bacterial infections, and evaluation of the prognosis of disease, has become a popular research focus.

Many researchers are focused on identifying accurate and quickly measured markers for monitoring of infectious diseases. A recently identified infection-related biomarker, procalcitonin (PCT) has been measured in a clinical setting, and this strategy has been effective in diagnosing infection at an early stage, in grading severity of infection, and in enabling prognostic assessment [5–8]. Some researchers believe that PCT can be used as an early indicator of infection in patients with acute leukemia during the agranulocytosis period [9], while others have argued that the significance of PCT remains unclear at this stage [10]. Therefore, we believe it is necessary to further investigate the clinical

Received • Примљено: August 11, 2016
Accepted • Прихваћено: November 22, 2016
Online first: March 14, 2017

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significance of PCT in providing a basis for subsequent anti-infective therapy and in assessing patients' clinical condition, in patients with acute leukemia who acquire bacterial infections during the agranulocytosis period post-chemotherapy. This study was conducted in accordance with the declaration of Helsinki, with approval from the Ethics Committee of the People's Hospital of Lishui City and the Sixth Affiliated Hospital of Wenzhou Medical University. Written informed consent was obtained from all participants.

The aim of the study was to investigate the clinical value of PCT in granulocyte deficiency with bacterial infection of patients with acute leukemia after chemotherapy, which provides evidence for clinical anti-infection treatment and disease assessment.

METHODS

Subjects

A total of 92 patients with acute leukemia (48 males and 44 females; 12–65 years old, with median age of 38.5 years) who were admitted to the Department of Hematology of the People's Hospital of Lishui City and the Sixth Affiliated Hospital of Wenzhou Medical University from July 2009 to December 2013 were enrolled in this study.

The inclusion criteria were the following: the age of the patients' was 12–65 years; the fever occurred in pro-chemotherapy agranulocytosis period; the first blood culture was bacteria-positive or negative, but clinical symptoms indicated infection; cytomegalovirus, Epstein–Barr virus, herpes simplex virus, varicella zoster virus and glucan negative tests were negative.

The exclusion criteria were as follows: fungal infection indicated by the pathogen culture and chest CT; virus infection indicated by the serological examination; transfusion associated fever; persistent fever without infection due to use of high-dose cytarabine. According to the hematopoietic and lymphoid tissue tumor classification standard from WHO, there were 68 patients with acute myeloid leukemia (AML) and 24 patients with acute lymphoblastic leukemia (ALL). Sixty-eight AML patients consisted of eight t(8;21) cases, 12 inv(16) cases, seven t(16;16) cases, eight t(15;17) cases, and 33 cases without specific classification (M1, 3; M2, 12; M4, 13; M5, 5). The 24 ALL patients included 19 cases of B-ALL and five cases of T-ALL. All the patients were cases with complete bone marrow remission after chemotherapy. The intensive treatment scheme for AML patients included HDAra-C, IDA, ID-Ara-C+Mit, ID-Ara-C+VP16, etc., and that for ALL patients included HDAra-C+HD-MTX, HD-MTX+VP, CDOLP, etc.

All the patients were at the agranulocytosis period post-chemotherapy and had acquired bacterial infections. The diagnosis of neutropenic fever, infection and severe sepsis was as follows: (1) neutropenic fever (neutropenia was defined as neutrophil count of $< 0.5 \times 10^9$/L, or count of $< 1 \times 10^9$/L with a predicted decrease to $< 0.5 \times 10^9$/L; the fever was defined as a single oral temperature of ≥ 38.5°C or a temperature of ≥ 38°C for ≥ 1 hour) [11]; (2) bacterial infection (the single oral temperature was ≥ 38.5°C or the body temperature was ≥ 38°C for ≥ 1 hour; the blood culture suggested bacterial infection, with or without clinical infection sign, defined as bacteremia; for blood culture negative patients, there were clinical infection signs such as cough, diarrhea, abdominal pain, urine or anal pain; the patients had an unexplained drop in blood pressure or blood oxygen saturation. Computed tomography, X-ray, and abdominal B ultrasound indicated a new infection focus; antibiotic treatment was effective; fungus and virus infections were excluded); (3) severe sepsis (the sepsis was associated with organ dysfunction, hypoperfusion or hypotension; perfusion abnormalities included, but were not limited to, lactic acidosis, oliguria, or acute alteration in mental status [12]).

Based on blood culture results, specimens were divided into a positive group (n = 30), and a negative group (n = 62). Positive blood culture referred to patients with at least one positive blood culture result; negative blood culture referred to patients who had negative blood culture results, but had the signs and symptoms of bacterial infection, as well as positive imaging findings for bacterial infection, and were sensitive to antibiotics. Bacteriological examination was performed using the VITEKAMS60 automatic bacterial analyzer (BioMerieux, Lyon, France). Among the positive blood culture group, specimens were further divided into a Gram-positive bacteria subgroup (n = 14), and a Gram-negative bacteria subgroup (n = 16).

Before chemotherapy, all the patients were given tests in routine blood, routine urine, liver and kidney function, C-reactive protein, PCT, cardiac ultrasound, hepatobiliary and urinary B ultrasound, chest computed tomography, hepatitis B virus, cytomegalovirus, Epstein–Barr virus, herpes simplex virus, and varicella zoster virus. If there was no abnormality, intensive therapy was performed. After the emergence of agranulocytosis, respiration, pulse, blood pressure, and peripheral oxygen saturation were daily monitored for revealing the potential infection focus. For the occurrence of fever, blood culture was conducted, as were other cultures, such as urine and throat swab culture. The routine tests were performed again. Finally, broad-spectrum antibiotic treatment was administered. The evaluation standard of curative effect was as follows: after using antibiotics for three to five days, the patient's body temperature gradually returned to normal, and vital signs gradually grew stable. The disappearance of clinical symptoms suggested that the patient was sensitive to antibiotics. After the white blood cells recovered to normal, the computed tomography, X-ray, and abdominal B ultrasound were performed to re-examine the new infection focus. The obvious remission or disappearance of infection focus presented the cure.

PCT measurement

Venous blood (2 ml) from patients with neutrophil counts $\leq 0.5 \times 10^9$/L and a single oral temperature of ≥ 38.5°C or axillary temperature of ≥ 38°C for ≥ 1 hour were collected before using antibiotics (D0) for serum isolation. Serum PCT levels were determined within four hours by using colloidal gold technology and detection with the LIAISON

automated fluorescence immunoassay analyzer (Sorin, Modena, Italy). Values of ≥ 0.5 ng/ml were regarded as cut-off.

Blood culture

Venous blood (16–20 ml) from patients with neutrophil counts of ≤ 0.5×10^9 /L and single oral temperature of ≥ 38.5°C or axillary temperature of ≥ 38°C for ≥ 1 hour were collected at D0 for two sets of blood culture (the interval between them was within 5 minutes). Each set of blood culture included one bottle of anaerobic bacteria and one bottle of aerobic bacteria.

Statistical analysis

Statistical analysis was performed using SPSS 12.0 statistical software (SPSS Inc., Chicago, IL, USA). Comparisons of serum PCT between the groups were carried out using the χ^2 test.

RESULTS

Treatment results

All the patients were given conventional treatment with broad-spectrum antibiotics. For some patients the antibiotics were adjusted according to drug sensitive test results. Five patients died due to severe sepsis during the treatment. Other patients were cured according to the efficacy evaluation standard.

Relationship between blood culture results and serum PCT

The median blood neutrophil absolute value of 92 patients was $0.03 \pm 0.16 \times 10^9$ /L. In 92 specimens tested by blood culture, 30 specimens obtained from 30 patients were bacteria-positive (32.6%, 30/92), and 62 specimens obtained from 62 patients were bacteria-negative (67.4%, 62/92). The bacteria of the blood culture are shown in Table 1. On D0, 68 cases exhibited PCT ≥ 0.5 ng/ml, accounting for 73.9% of total patients. PCT ≥ 0.5 ng/ml was found in 87% of the bacteria-positive specimens, with 67.7% of bacteria-negative specimens ($p < 0.05$). The D0 serum PCT was negative in 13% of bacteria-positive specimens, with 32.3% of bacteria-negative specimens ($p < 0.05$). The expression intensity for serum PCT ≥ 0.5 ng/ml in the bacteria-positive group was 12.4 ng/ml, while it was 5.6 ng/ml in the bacteria-negative group. PCT in the bacteria-positive group was significantly higher than that in the bacteria-negative group ($p < 0.05$, Table 2).

Serum PCT levels in patients with different bacterial infections

Of the 30 specimens obtained from 30 patients that were bacteria-positive, 14 specimens showed Gram-positive bacterial infection (46.7%, 14/30), and 16 specimens showed Gram-negative bacterial infection (53.3%, 16/30). In serum PCT positive patients, the average serum PCT levels in the Gram-negative group was 14 ng/ml, Gram-positive group was 7.5 ng/ml, and the serum PCT levels in the Gram-negative group were significantly higher than those in the Gram-positive group ($p < 0.05$, Table 3).

Table 1. Bacterial species in 30 patients with positive blood culture

Bacterial species	Number of strains
Gram+ bacteria	14
Staphylococcus epidermidis	3
Staphylococcus aureus	4
Streptococcus viridans	2
Hemolytic streptococcus	2
Enterococcus faecalis	1
Rothia mucilaginosa	1
Enterococcus faecium	1
Gram- bacteria	16
Escherichia coli	3
Enterobacter cloacae	2
Pseudomonas maltophilia	2
Pseudomonas aeruginosa	2
Klebsiella pneumoniae	4
Fusobacterium	2
Morganella morganii	1

Table 2. Serum PCT levels in 92 specimens grouped by the blood culture results

Groups	cases (n)	PCT subgroup, No. and mean (ng/ml)	
		< 0.5	≥ 0.5
Bacteria-positive	30	4 (13%) 0.1	26 (87%) 12.4
Bacteria-negative	62	20 (32.3%) 0.11	42 (67.7%) 5.6
Total	92	24	68

Table 3. Serum PCT levels in the Gram-positive (G+) and Gram-negative (G-) infection groups

Groups	Cases (n)	PCT, No. and mean (ng/ml)	
		< 0.5	≥ 0.5
G-	16 (53.3%)	1 0.11	15 14
G+	14 (46.7%)	3 0.09	11 7.5
Total	30	4	26

The prognostic significance of serum PCT

To explore the prognostic significance of serum PCT levels for the patients with acute leukemia during the agranulocytosis period post-chemotherapy who had acquired bacterial infections we further analyzed data on serum PCT levels from patient specimens that were bacteria-positive. In this study, five patients died of infections, the blood culture of four patients was Gram-negative bacteria, and one case was Gram-positive bacteria.

The comparison of the remaining 25 patients with bacteria-positive specimens is as follows: in five cases of death, the average PCT value was 28.2 ng/ml; the patients who survived had the average PCT value of 12 ng/ml; serum

PCT levels in patients who died were significantly higher than those found in patients who survived ($p < 0.05$).

DISCUSSION

PCT constitutes 116 amino acids and has a molecular weight of 13 kDa [13]. PCT is cleaved in vivo into PCT and binding calcitonin, with binding calcitonin being further converted enzymatically into calcitonin, which plays a physiological role in vivo. In the event of infection, PCT levels increase hundred-fold. Thus, serum PCT measurement has been increasingly applied to the monitoring of infectious disease in a clinical setting [14, 15].

During the agranulocytosis period, post-chemotherapy patients with acute leukemia are at an increased risk of bacterial infections, the leading cause of death in these circumstances. Thus, early diagnosis and prompt treatment of infection is very important in patients' prognosis. Studies have shown that, with the cut-off value for PCT level set to 0.5 ng/ml, diagnostic sensitivity to bacterial infection was 65%, and specificity 96%. Additionally, at serum PCT levels higher than 1.2 ng/ml, sensitivity reaches 100% [16]. In many studies, 0.5 ng/ml is often used as the cut-off value of PCT [17]. We conducted this study according to the above reported methods and found that, patients with etiologically confirmed bacterial infections had significantly higher serum PCT, as did patients that were diagnosed clinically, but had negative blood culture results, especially for PCT-positive cases. This is consistent with other studies mentioned above. Our study found that, on the exact day of fever, 73.9% of the patients had PCT over the threshold, the PCT-positive-rate of the positive blood culture group (≥ 0.5 ng/ml) was higher than the negative blood culture group (87% vs. 67.7%, $p < 0.05$), indicating that the PCT value of the positive blood culture group was higher than the negative blood culture group. Our study also shows that patients infected with Gram-negative bacteria had significantly higher serum PCT levels than patients infected with Gram-positive bacteria ($p < 0.05$). In serum PCT-positive patients, the average serum PCT level in the Gram-negative group was 14 ng/ml, while the level in the Gram-positive group was 7.5 ng/ml. Furthermore, serum PCT levels in patients that died were also significantly higher than in those that survived; this indicates that measurement of serum PCT can be applied to the early diagnosis of infection and to the classification of the disease severity. These results are consistent with the findings of Jeddi et al. [18], Neofytos et al. [19], and Hatzistilianou et al. [20]. Our study has confirmed this conclusion. However, this is a single-center study. Due to a smaller sample size it needs to be further verified.

For patients with agranulocytosis who do not show obvious symptoms of infection, measurement of serum PCT at the early stages of infection will not only help to assess the infection severity and increase the efficacy of anti-infective therapy but also help to distinguish between Gram-negative and Gram-positive bacterial infection to provide a reasonable basis for the clinical administration of antibiotics [21, 22, 23]. For patients with acute leukemia acquiring bacterial infection during the agranulocytosis period post-chemotherapy, our findings are consistent with most other studies [24, 25]. We found that the PCT level in Gram-negative infected patients was higher than in Gram-positive infected patients. In five cases of death, the average PCT reached 28.2 ng/ml, and the average PCT level of these patients was higher than other positive blood culture patients. Therefore, we initially thought that the increasing level of PCT could reflect the severity of bacterial infection, especially in severely infected patients, in whom PCT was obviously increased. Our results initially considered that in the period of agranulocytosis combined with bacterial infection that occurred after the chemotherapy of acute leukemia, procalcitonin had a clinical significance in predicting bacterial infection, infected bacterial types, and severities. Combined with the previous studies, it could be used as a confirmed clinical examination index to guide the clinical judgment.

CONCLUSION

Our results showed that PCT of bacterial infection confirmed by etiology was significantly increased, while positive results of patients who were considered te be suffering from a bacterial infection with blood-free culture also increased. This indicates that PCT can be used not only in early diagnosis of diseases, but also in the classification of the severity of a disease. For patients with granulocyte deficiency who have no obvious symptoms and whose condition changes rapidly, the detection of serum PCT levels in early infection can not only make a judgment on the severity of infection and anti-infection effect, but can distinguish Gram-negative from Gram-positive bacteria, providing the basis for the reasonable application of antibiotics. For patients in granulocyte deficiency with bacterial infection with acute leukemia after chemotherapy, serum PCT levels have an important value for reflecting the extent of bacterial infection and the anti-infection treatment, which is a reliable index for monitoring and has important clinical value.

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Прокалцитонин као показатељ бактеријске инфекције у постхемиотерапијској агранулоцитози код акутне леукемије

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САЖЕТАК

Увод/Циљ Бактеријска инфекција због мањка гранулоцита је последица хемиотерапије акутне леукемије и водећи је узрок смрти. Тренутно постоји мали број довољно осетљивих показатеља који би указивали на бактеријску инфекцију и који немају јасну одређеност за постављање дијагнозе инфекције. У више студија прокалцитонин (ПКТ), прекурсор калцитонина, показао се као брз и сигуран показатељ инфекције, али је његов клинички значај нејасан.
Циљ овог рада је био да се одреди клинички значај нивоа ПКТ-а код болесника са акутном леукемијом и инфекцијом услед постхемиотерапијске агранулоцитозе.

Методе Ниво ПКТ-а у серуму је анализиран код 92 болесника са акутном леукемијом и инфекцијом услед постхемиотерапијске агранулоцитозе.

Резултати Ниво ПКТ-а у серуму болесника са позитивном хемокултуром је значајно виши него код болесника са негативном хемокултуром ($p < 0.05$). Грам-негативне бактерије су биле значајно чешћи узрочник инфекције од грам-позитивних ($p < 0.05$). Осим тога, болесници са позитивном хемокултуром и повишеним ПКТ-ом су значајно чешће умирали него преживљавали ($p < 0.05$).

Закључак У периоду агранулоцитозе са бактеријском инфекцијом услед хемиотерапије акутне леукемије ПКТ може указати на бактеријску инфекцију, врсту и тежину инфекције

Кључне речи: прокалцитони; акутна леукемија; бактеријска инфекција; агранулоцитоза

DOI: https://doi.org/10.2298/SARH160901068R
UDC: 616.921.5-035.22(497.113)"2010/2015"

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Surveillance of influenza in the post-pandemic period in Vojvodina, Serbia, October 2010 – May 2015

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SUMMARY

Introduction/Objective In August 2010, World Health Organization declared the beginning of the post-pandemic phase of influenza surveillance.
The aim of this study was to evaluate the epidemiological and virological characteristics of influenza and correlation between the influenza occurrence and weather conditions.

Methods We used surveillance reports of influenza and laboratory data from October 2010 to May 2015. Data for the analysis were collected through sentinel surveillance of influenza-like illness (ILI), severe acute respiratory illness (SARI), acute respiratory distress syndrome, and by virological surveillance. The nasal and throat swabs from all influenza cases were performed by the PCR laboratory method.

Results During the observed period, the highest rates of ILI were registered during the 2010/11 and 2012/13 seasons, with influenza A (H1N1)pdm09 and influenza B being predominant, respectively. The highest weekly age-specific rates of ILI were registered in school-age children (ages 5–14). Out of 1,466 samples collected, 720 (49.1%) were laboratory confirmed as influenza, and influenza A virus was more frequently detected than influenza B. Among confirmed cases of influenza, participation of patients with SARI or ILI was nearly equal (46% vs. 44.1%). There was a weak correlation observed between the decrease in temperature and rainfall and the increase in influenza detection ($\rho = -0.04214$ vs. $\rho = -0.01545$, respectively, $p > 0.05$).

Conclusion There is a need for continuous surveillance in order to predict seasonal trends and prepare for a timely response to influenza outbreak.

Keywords: influenza virus; epidemiology; virology; sentinel surveillance

INTRODUCTION

Increased influenza's viral activity causes a high incidence, exceeding incidence of other infectious diseases [1]. The results of epidemiological surveillance of influenza are important as they provide the information which serve as the early warnings of epidemics, and give us an insight into the type and/or subtype of influenza virus, so we can prepare the adequate influenza vaccines and antiviral drugs. They are also significant for implementing appropriate measures for reducing the number of deaths caused by influenza, hospitalization rates due to complications, and the costs of the treatment of influenza [1, 2, 3].

The main factors which determine the seasonality of influenza are still unclear, especially in the tropical areas of the world. Probably, the combined action of environmental factors (humidity, temperature), factors related to immunity of the population, and demographic factors (population density, population flows, school calendar, and traveling or migration) interfere with viral circulation in different parts of the world [4, 5].

Surveillance of influenza at the European level has been conducted since 1996 [6]. In order to improve the monitoring of the epidemiological situation, to ensure efficient response and to reduce the negative consequences on the health of the population, sentinel surveillance of influenza like illness (ILI) and acute respiratory infection (ARI) was introduced in the Autonomous Province of Vojvodina (the northern region of Serbia) in the 2004/05 season, as a pilot study, according to the model of surveillance conducted in Slovenia [7]. In the preparation for an influenza pandemic, sentinel surveillance in Vojvodina became the standard model and also a part of a newly established national influenza surveillance, throughout the Republic of Serbia, since 2009. Due to the quality of the results of the surveillance of influenza in Vojvodina, special Public Health Program was introduced for surveillance of ILI/ARI, severe acute respiratory illness (SARI), and acute respiratory distress syndrome (ARDS) [8]. Similar to other countries in Europe, Vojvodina, as part of Serbia, has a reference laboratory for influenza. In this way, virological surveillance as an integral part of

Received • Примљено: September 1, 2016
Revised • Ревизија: October 27, 2016
Accepted • Прихваћено: November 7, 2016
Online first: March 14, 2017

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Table 1. Number of observed inhabitants during the sentinel surveillance of influenza-like illness by five seasons in the Autonomous Province of Vojvodina

Age groups / Seasons	0–4	5–14	15–29	15–64	30–64	≥ 65	Total	Number of included sentinel physicians
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
2010/11	9,281 (10.5)	15,563 (8.2)	-	47,301 (3.5)	-	27,413 (8.7)	99,558 (5.2)	93
2011/12	9,843 (11.1)	17,857 (9.5)	-	60,001 (4.5)	-	25,565 (8.1)	113,266 (5.9)	94
2012/13	10,388 (11.7)	19,837 (10.5)	23,000 (6.3)	-	55,418 (5.7)	27,651 (8.7)	136,294 (7.1)	89
2013/14	23,664 (26.7)	61,157 (32.4)	56,303 (15.5)	-	102,271 (10.5)	48,352 (15.3)	304,719 (15.8)	135
2014/15	17,064 (19.2)	33,298 (17.6)	34,609 (9.5)	-	66,152 (6.8)	33,420 (10.6)	184,543 (9.6)	135

n – surveillance population by age groups and seasons;
% – percentage of population in certain age group according to the census

epidemiological surveillance provides useful information about circulation of an actual influenza virus [9].

In August 2010, the World Health Organization (WHO) declared the post-pandemic period [10]. However, influenza A (H1N1) pdm09, A (H3N2), and B viruses have continued to circulate in the population after the pandemic period [11].

The main goal of this study was to observe seasonal influenza activity along with influenza occurrence and weather conditions in Vojvodina, based on data collected between October 2010 and May 2015, in the post-pandemic period (five surveillance seasons).

METHODS

Data for this observational study were obtained from the sentinel surveillance of ILI, surveillance of all hospitalized patients with SARI and/or ARDS, and virological surveillance of influenza in Vojvodina through the whole season (from calendar week 40 of each year to calendar week 20 of the next year). All information about patients was anonymized and de-identified. Data on temperature and average rainfall were provided by the Republic Hydrometeorological Service of Serbia.

Sentinel surveillance of ILI

During the seasons of surveillance of ILI and ARI, from 2010 to 2015, sentinel surveillance was conducted in all seven districts of Vojvodina. In the surveillance of ILI and ARI, only outpatients in Health Centers of Vojvodina were covered. In accordance with the WHO and national recommendations, the surveillance of influenza was conducted within four age groups (0–4, 5–14, 15–64, and ≥ 65 years) during the 2010/11 and 2011/12 seasons. Since the 2012/13 influenza season, the surveillance has included five age groups (0–4, 5–14, 15–29, 30–64, and ≥ 65 years). Between the 2010 and 2013 seasons, sentinel surveillance has been implemented in 19 municipalities with more than 30,000 inhabitants, encompassing between 5.2% and 7.1% of the total population. During the last two seasons (2013–2015), sentinel surveillance of influenza was extended to municipalities with less than 30,000 inhabitants and covered all of 45 municipalities of the Province (and included 135 sentinel physicians). Although the number of sentinel physicians during the two last seasons was the same, because we included different sentinel physicians, the observed populations were not equal (Table 1).

The surveillance system used the network of sentinel physicians (general practitioners and pediatricians) who reported the number of new cases of ILI in their reference populations weekly, and collected respiratory samples for virological evaluations. In addition, sentinel physicians electronically entered the data of new cases of ILI and ARI by week, and regularly sent samples for virological confirmation to the WHO National Influenza Center, the Center of Virology of the Institute of Public Health of Vojvodina in Novi Sad. Each week, global population was adjusted with ILI and ARI rate for Vojvodina, which allows to follow the indicators of influenza activity [7]. The influenza surveillance data were collected for the whole season (from calendar week 40 of each year to calendar week 20 of the following year) [12].

SARI and ARDS surveillance

Since the 2010/11 influenza season, in accordance with the WHO recommendations, SARI surveillance has been carried out during the winter season only, from week 40 to week 20 [13].

As described in detail previously, hospital coordinators for SARI and ARDS surveillance from all 15 acute care hospitals in Vojvodina sent daily reports on every hospitalized SARI and/or ARDS case to the district coordinators of influenza in the local departments of Public Health [7]. The study included patients from intensive care units and high dependency units (severe form of infections), general/internal medicine, pediatric medicine, infectious disease wards and respiratory disease wards where patients of all ages were registered [8].

Case definitions

This study included all patients with ILI and any flu-associated infections who required hospitalization in accordance

with the SARI and ARDS definitions. The WHO criteria for ILI and SARI were used for screening in the primary care/outpatient settings and inpatient hospital settings: ILI cases were defined as those with a sudden onset of fever (> 38°C) and cough/sore throat within seven days of the onset, while patients with an acute respiratory illness with the onset of symptoms during the previous seven days requiring overnight hospitalization which included history of fever or measured fever of 38°C, and cough, and shortness of breath or difficulty in breathing were termed as SARI [14].

ARDS cases were defined as acute onset of bilateral infiltrates on the chest radiograph; arterial oxygen tension (PaO_2)/fraction of inspired oxygen (FiO_2) ratio < 27kPa, and absence of a cardiac failure or left atrial hypertension (assessed clinically, echocardiographically, or with invasive monitoring) and required invasive ventilation [15, 16, 17].

Virological surveillance

The reference laboratory for virological surveillance of influenza in Vojvodina is the WHO National Influenza Center at the Institute of Public Health of Vojvodina, Novi Sad [18].

Nasal and throat swabs from patients with suspected influenza were placed in the same vial with transport medium and kept at -20°C. The transport of the samples to the laboratory was organized on a daily basis by local departments of public health. Viral RNA was extracted using the commercially available QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The reverse transcription and amplification were performed using the AgPath-IDTM One-Step RT-PCR Reagents (Applied Biosystems, Foster City, CA, USA) on a 7500 Real-Time (Applied Biosystems) thermocycler. Influenza A and B virus detection (without further determination of B/Yamagata-like and B/Victoria-like viruses) was done by singleplex real-time RT-PCR assays. The testing was done according to the protocol developed by the Centers for Disease Control and Prevention, enclosed with the reagents. The results were analyzed using the Applied Biosystems 7500 Software version 2.0.6, and the interpretation of the data was done according to the WHO guidelines [19]. Immediately after the testing was finished, the results of the laboratory tested samples were sent to the Institute of Public Health of Serbia in Belgrade, local departments of public health, the sentinel/hospital physician, and to the patients [7].

Statistical analysis

Similar to the previously used methodology, the population under surveillance was used as a denominator for calculations of the weekly incidence of ILI and the numerator was the number of clinical cases of ILI in the total population and in the age groups [7]. Also, the weekly age-specific incidences of ILI for monitored age groups were measured per 100,000 inhabitants.

The epidemic threshold of incidence of 246.3/100,000 was determined in the previous five pre-pandemic sentinel seasons on the basis of the weekly incidence rate of ILI value [7].

A correlation analysis was performed using the Spearman correlation coefficient between the detected monthly influenza cases and the average monthly temperature and rainfall using the data from the Republic Hydrometeorological Service of Serbia, for the capital of the Autonomous Province of Vojvodina, city of Novi Sad [20]. Because the Meteorological annual data from the Republic Hydrometeorological Service of Serbia contains the average monthly values for only six different meteorological stations (regions) in Serbia (Belgrade, Novi Sad, Vranje, Zlatibor, Loznica, and Niš), we used values of average monthly temperature and rainfall only for the city of Novi Sad (Vojvodina), which represents weather conditions for this region of Serbia. Average temperature and rainfall were measured in degrees Celsius (°C) and millimeters (mm), respectively.

Two tailed p-values less than 0.05 were considered statistically significant.

RESULTS

Surveillance of ILI

During the study period (2010–2015), the highest weekly incidence rates were between 474.5/100,000 (2010/11 season, with influenza A (H1N1)pdm09 being predominant) and 712.3/100,000 (2012/13 season, with influenza B being predominant). The lowest weekly incidence rate was registered during the 2013/14 season, predominated by influenza A (H3N2). In the 2010/11 influenza season, with the predominance of influenza A (H1N1)pdm09 (ILI incidence above the epidemic threshold of 246.3 cases per 100,000 population) the duration of the epidemic period was eight weeks. Only during the 2013/14 season, values of the weekly ILI incidence were below the epidemic threshold (Figure 1).

Figure 2 shows the trend of ILI weekly incidence rate by age group during the five post-pandemic seasons. During the observed period, it was evident that the highest weekly age-specific incidence of ILI was registered in school-age children (5–14 years old), with the highest incidence rate

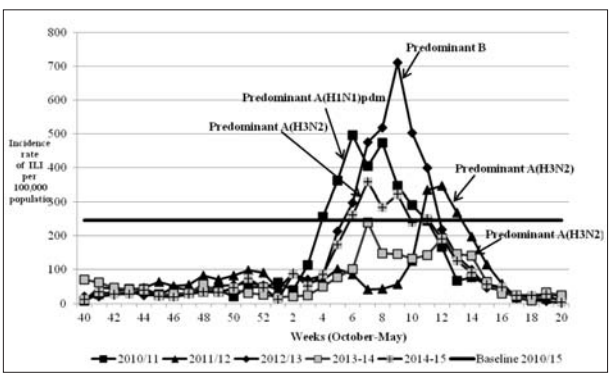

Figure 1. Influenza-like illness (ILI) in the 2010–15 seasons according to the influenza sentinel surveillance system in the Autonomous Province of Vojvodina

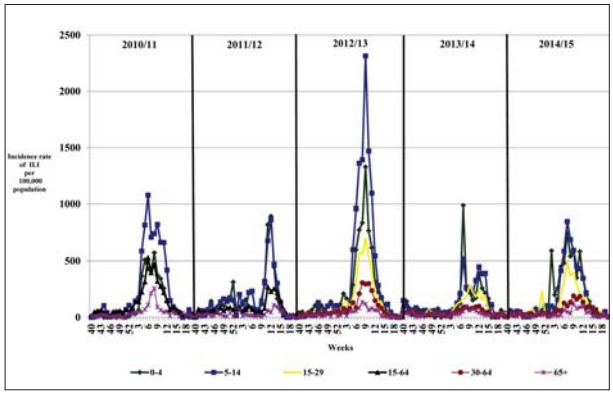

Figure 2. Age-specific incidence of ILI per 100,000 population in the Autonomous Province of Vojvodina during five influenza seasons; black vertical lines indicate the separation between different influenza seasons (2010–15)

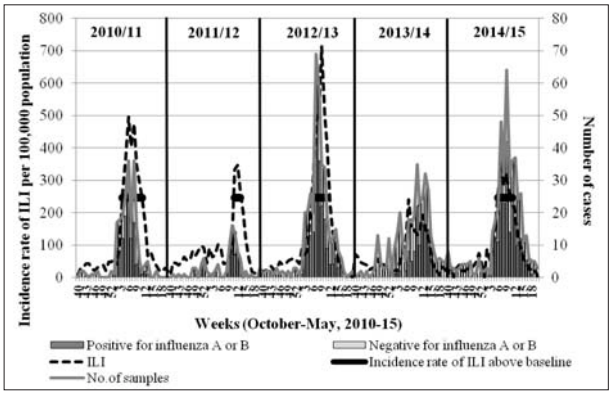

Figure 3. Incidence rate of ILI consultations per week by case status for the sentinel surveillance network, in the Autonomous Province of Vojvodina, during 5 influenza seasons; black vertical lines indicate the separation between different influenza seasons (2010–15)

Table 2. Distribution of influenza infection by case definitions and by type/subtype in the Autonomous Province of Vojvodina, 2010–15 seasons

Variable		2010/11		2011/12		2012/13		2013/14		2014/15		Total	
		n	%	n	%	n	%	n	%	n	%	n	%
Clinical form	ILI	29	24.6	49	92.5	72	36.2	63	53.8	105	45.1	318	44.1
	SARI	66	55.9	4	7.5	110	55.3	47	40.2	104	44.6	331	46
	ARDS	23	19.5	0	-	17	8.5	7	6	24	10.3	71	9.9
Influenza virus type/subtype	A (H1N1)pdm09	101	85.6	0	-	68	34.2	33	28.2	86	36.9	288	40
	A (H3N2)	6	5.1	52	98.1	28	14.0	77	65.8	90	38.6	253	35.1
	A (non-subtype)	3	2.5	0	-	2	1.0	7	6	9	3.9	21	3
	B	8	6.8	1	1.9	101	50.8	0	-	48	20.6	158	21.9
Total		118	100	53	100	199	100	117	100	233	100	720	100

ILI – influenza-like illness, SARI – severe acute respiratory infection; ARDS – acute respiratory distress syndrome

of 2,315.2/100,000 in the 9/2013 week. In all five seasons, the lowest weekly age-specific incidence of ILI was registered in the oldest population.

In all five influenza seasons, the highest value of weekly ILI incidence rate was accompanied by the highest number of suspected and confirmed influenza cases (Figure 3).

Influenza by case definitions and prevalence distribution of influenza viruses

In the observed period, 1,466 specimens from patients with ILI, SARI, or ARDS, were tested, and 720 samples were identified as influenza A or B positive (49.1%). There were a total of 562 influenza A and 158 influenza B virus infections confirmed during the five influenza seasons. During all five seasons, influenza A (H1N1)pdm09 was predominant (40%, 288/720). Participation of patients with SARI or ILI was nearly equal (46%, 331/720 vs. 44.1%, 318/720, respectively). Patients with ARDS were not registered only during the 2011/12 influenza season (Table 2).

Weather conditions and prevalence of influenza

The dependence of influenza activities on weather conditions in the Province (meteorological station for the city of Novi Sad) during the investigation period is shown in Figure 4. During three influenza seasons (2011/12, 2012/13, and 2014/15), the highest number of confirmed influenza infections was registered during March. The average monthly temperature (October–April, 2010–15) was between -4.9°C and 13.6°C, and average values of rainfall in the same period were 1.3–82.8 mm. These data show that the average monthly temperature and average rainfall do not have any significant correlation with the number of influenza-positive cases (ρ = -0.04214 vs. ρ = -0.01545; $p > 0.05$, respectively).

DISCUSSION

We presented the first description of the epidemiological and virological characteristics of influenza and the correlation between the influenza occurrence and weather conditions after the pandemic 2009/10 season in Vojvodina. As can be concluded from the obtained data, all influenza viruses were detected across Vojvodina and all age groups were affected during the post-pandemic seasons.

During the 2010/11 season in Western Europe, transmission peaked during late January and early February, and two to three weeks later in Eastern Europe, with influenza A (H1N1) virus as predominant in 2009. Opposite Europe, where influenza A (H3N2) was rare, it was predominant in North America. During this season, in several countries, a higher number of severe cases of influenza were registered compared to the previous season, but reasons for this were not clear [21]. As it was expected, influenza A(H1N1)pdm09 virus predominated among the vi-

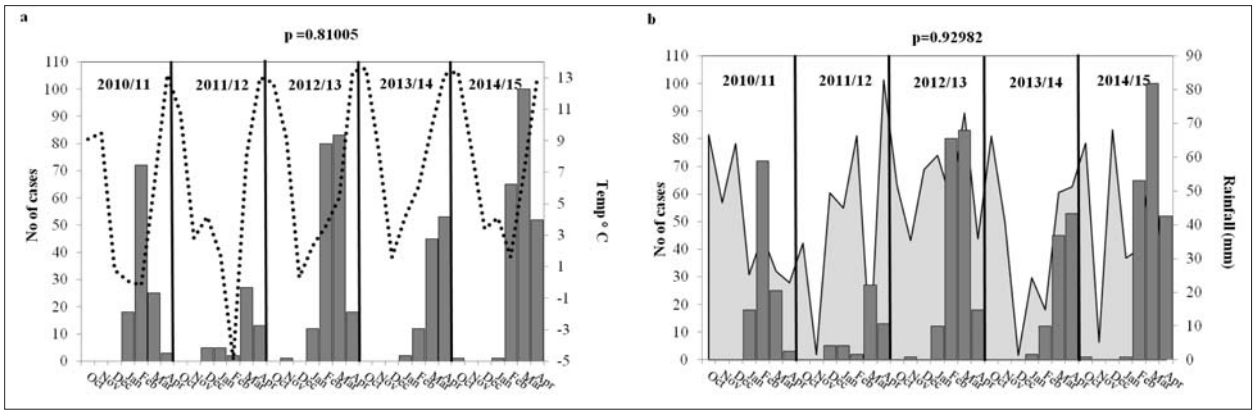

Figure 4. Correlation of the number of influenza cases in the Autonomous Province of Vojvodina in the investigated period with: (a) the average temperature (°C); (b) the average rainfall (mm); black vertical lines indicate the separation between different influenza seasons (2010–15)

ruses detected during the first post-pandemic season, but the results of the phylogenetic analysis of HA sequences of A(H1N1)pdm09 viruses circulating in Lombardy showed that viruses isolated during the 2010/11 season segregated into a different genetic group with respect to those identified during the 2009 pandemic [22].

In Vojvodina, during the 2010/11 season, similar to the previous season, influenza A (H1N1)pdm09 was predominant (85.6%, 101/118), with peaked transmission during late January and throughout all of February. The highest incidence rate of ILI was registered in the 5–14 years age group. The epidemic wave (incidence rate of ILI above 246.3/100,000 population) lasted for eight weeks, and as much as 75.4% (89/118) of all confirmed cases of influenza were hospitalized for SARI or ARDS.

Similar to the results of the WHO review, in Vojvodina, during the 2011/12 season, influenza transmission peaked during late March [23]. Influenza A (H3N2) was predominant 98.1% (52/53), and the highest incidence rate of ILI was registered among the youngest patients and in the school-age children (0–4 and 5–14 years old). The epidemic wave lasted for only three weeks.

In North America, the 2012/13 influenza season started earlier than in other parts of the Northern Hemisphere. In Europe, the influenza season peaked two weeks later than in North America, and it was also unusually long, associated with a late rise in influenza B cases in many countries. Peaking of influenza activity was registered around week 5/2013 and lasted until week 16/2013. In North America and Europe, later in the season, influenza B type was more commonly detected than either of the influenza A subtypes [24, 25].

In Vojvodina, the highest incidence rate of ILI was registered in the 9/2013 week (712.3/100,000 population), and the epidemic wave lasted for six weeks (weeks 6–11). Among confirmed cases of influenza, the predominant type was influenza B (50.8%, 101/199).

In the 2013/14 season in Europe, influenza activity started later than usual, with a very small increase during the final weeks of December, followed by more marked increase throughout January. Overall activity continued to increase through February. Influenza activity during the 2013/14 season was less intense, with fewer positive samples detected than in the previous post-pandemic seasons. Overall, influenza A (H1N1)pdm09 was predominant in most of northern European countries and A (H3N2) was predominant in most of eastern European countries. Unlike North America, where influenza B was detected in the late season, the detection of influenza B in Europe remained low during the entire season [26].

In our territory, during the 2013/14 influenza season, the first influenza case was registered in the 2/2014 week, and the incidence rate of ILI was below the baseline for the entire season. Similarly to the 2011/12 influenza season, the highest incidence rate of ILI was registered among the youngest and in the school-age children. We think that the reasons for the highest incidence rates of ILI among the mentioned age groups perhaps lie in the fact that young people are tested more often [7]. Furthermore, as is well known, children may shed viruses more profusely and for longer periods of time than adults [19].

Similarly to the results for Eastern Europe, the predominant subtype of influenza in Vojvodina was A (H3N2), with 65.8% (77/117) of all confirmed cases [26]. Unlike the previous season, when influenza B was the predominant type, it was not detected among the tested samples during the 2013/14 season.

Considering European countries, the timing of influenza detections during the 2014/15 season was similar to the previous years. The influenza activity started increasing in the last few weeks of 2014, similar to the situations in the 2013/14 and 2011/12 seasons. The peak of influenza activity in 2015 varied between countries, but it was most often between weeks 6 and 9. However, the percentage of positive influenza cases was still above the threshold in many countries in April. During the 2014/15 season, influenza A (H3N2) virus was predominant in most regions, and an increased proportion of influenza B virus was registered after the activity peak. During this influenza season, most of circulating influenza A (H3N2) viruses were different from the virus used in vaccines in the northern hemisphere [27, 28].

The data obtained from this study showed that during the 2014/15 season, epidemic influenza activity started in the 6/2015 week and lasted to the 11/2015 week. Like in previous seasons, the lowest weekly age-specific

incidence of ILI was registered in the oldest population. This phenomenon can be interpreted by the fact that older patients are more likely to be registered in the hospital institutions than at the primary care level (sentinel surveillance of influenza). During the last influenza season, the predominant virus was A (H3N2), with 38.6% (90/233), whereas the registered proportion of influenza B was 20.6% (48/233) of all confirmed cases. The last cases of influenza were registered on April 30, 2015.

In terms of seasonal pattern, in the Northern Hemisphere, influenza viruses are more frequently detected in autumn, winter, and spring, with the peak of influenza activity occurring typically between December and March and lasting for six to eight weeks. In temperate regions of the Southern Hemisphere, influenza activity typically peaks between May and September. In addition, influenza viruses can be isolated sporadically throughout the year [19].

In countries with tropical climate, influenza activity has been reported to peak in the rainy season, between June and August [29].

The climate in our country can be described as moderate continental, with intermediate temperatures and relatively low humidity. June is the rainiest, with the average of 12–13% of total annual precipitation sum. February and October have the least of precipitation [20]. During the study period (from the beginning of October to the end of April, 2010–2015) there was no significant correlation between the number of influenza detections by months and average monthly temperature and rainfall.

Although almost 55% of all confirmed cases of influenza were recorded in the city of Novi Sad, absence of data on monthly average temperature and rainfall in other parts of the Province and their comparison with influenza cases may be considered to be a limitation of this study.

CONCLUSION

The findings of the presented sentinel ILI and SARI surveillance, along with the virological surveillance, which are comparable with the results of surveillance of influenza in other countries across Europe, offer the possibility to determine the epidemiology of influenza during the post-pandemic seasons and predict future epidemic occurrences of influenza in Vojvodina. Likewise, the increasing number of samples among some age groups (0–4- and 5–14-year-olds) with the highest value of weekly ILI incidence rate would lead to a more precise view on the distribution of influenza in Vojvodina in the younger population.

ACKNOWLEDGMENTS

The authors are grateful to all physicians who participated in the influenza surveillance system in Vojvodina during the 2010–2015 seasons.

NOTE

This investigation was carried out as part of routine activities without additional funding sources.

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Надзор над инфлуенцом током постпандемијског периода у Војводини, од октобра 2010. до маја 2015.

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САЖЕТАК

Увод/Циљ У августу 2010. године Светска здравствена организација је прогласила почетак постпандемијске фазе у надзору над грипом.

Циљ рада био је да се процене епидемиолошке и вирусолошке карактеристике грипа и однос између климатских услова и преваленције грипа у постпандемијском периоду.

Методе Коришћени су подаци из надзора над грипом и резултати лабораторијских испитивања у периоду од октобра 2010. до маја 2015. године. Подаци су прикупљени кроз сентинелни надзор за обољења слична грипу (ОСГ), тешку акутну респираторну болест (ТАРБ), акутни респираторни дистрес синдром (АРДС) и вирусолошким надзором. Сви случајеви грипа потврђени су из назофарингеалног бриса лабораторијском методом *PCR*.

Резултати У посматраном периоду највише стопе ОСГ регистроване су током сезоне 2010/11. и 2012/13, када су доминирали вирус грипа типа А (*H1N1*)*pdm09*, односно вирус грипа типа Б. Највише вредности узрасно специфичних стопа ОСГ регистроване су у школском узрасту (5–14 година). Од 1.466 тестираних узорака, лабораторијска потврда вируса грипа добијена је код 720 (49,1%), а вирус грипа типа А чешће је детектован од вируса грипа типа Б. Међу потврђеним случајевима грипа учешће оболелих са дијагнозама ТАРБ или ОСГ је било скоро подједнако (46 тј. 44,1%). Није утврђена статистички значајна разлика између смањења температуре и падавина у односу на пораст броја потврђених случајева грипа (ρ = -0,04214 наспрам ρ = -0,01545, $p > 0,05$).

Закључак Наставак надзора над грипом кроз процену сезонских трендова омогућава спремност на одговор у случају појаве епидемије грипа.

Кључне речи: вирус грипа; епидемиологија; вирусологија; сентинелни надзор

DOI: https://doi.org/10.2298/SARH170220073P
UDC: 616.12-006-053.31

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Echogenic cardiac mass in the left atrium and associated supraventricular tachycardia in a neonate

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SUMMARY

Introduction Primary cardiac tumors in children are rare and the majority of them are diagnosed before the age of one year. They are mainly rhabdomyomas and have a tendency to regress. The incidence of arrhythmias is not well-defined, depending on the size and location of tumors.

Case outline The authors report a female neonate with ongoing fetal supraventricular tachycardia (SVT). Delivery by urgent cesarean section was performed with neither fetal echocardiography nor fetal antiarrhythmic drug intervention. Electrocardiogram confirmed tachycardia with narrow QRS complex at a rate of 260 beats/min. converting to sinus rhythm after a third dose of intravenous bolus injection of adenosine-5'-triphosphate. But the rhythm reverted to SVT showing refractory supraventricular reentrant tachycardia. Echocardiography performed after conversion to sinus rhythm showed an echogenic, well circumscribed mass in the left atrium, fixed to the primum atrial septum without other structural defects. SVT was treated by a bolus of amiodarone followed by an intravenous infusion. Long-term management with oral amiodarone and beta blocker had a good response. During the one-year follow-up echocardiograms were performed every month showing complete regression of cardiac mass, and there has been no recurrence of tachycardia since neonatal period.

Conclusion Tumor regression and a good long-term outcome in our patient suggest that it was probably a small but unfavorably positioned rhabdomyoma, associated with fetal and perinatal SVT. Prognosis and outcome of the disease depends on timely diagnosis and prompt and adequate treatment of associated life-threatening arrhythmias.

Keywords: cardiac tumor; supraventricular tachycardia; rhabdomyoma; neonate

INTRODUCTION

Primary cardiac tumors are rare in fetuses and neonates, with an incidence ranging between 0.0017% and 0.28 % [1]. Most of these tumors are benign, although sometimes their localization and clinical manifestation can be malignant [2]. Others, in the absence of hemodynamic changes, remain undiagnosed and spontaneously regress with time [3]. Histologically, the most common tumor in the neonatal age is rhabdomyoma [4]. If tumors are multiple, associated eventual tuberous sclerosis should be considered [5]. As rhabdomyomatous tissue can generate myocardial electrical potential and act as an accessory pathway, arrhythmias may develop as the main symptom, even in a fetus, where it could cause hydrops fetalis. Persistent arrhythmia in postnatal life increases the risks of neonatal death and these patients require prolonged anti-arrhythmic therapy [6]. Arrhythmia can sometimes disappear with spontaneous regression of the tumors [5]. Tachycardias most commonly associated with cardiac tumors described in the literature are ventricular tachycardia and supraventricular tachycardia (SVT) [2, 7].

The authors report a neonate with an ongoing fetal SVT and left atrium cardiac mass detected after birth.

CASE REPORT

A two-hour-old newborn was admitted to the neonatal intensive care unit after emergency cesarean section due to antenatal diagnosed SVT at the gestational age of 36/37 weeks. SVT was accidentally discovered during a routine control examination of pregnant primipara women. Delivery by urgent cesarean section was performed with neither fetal echocardiography nor fetal antiarrhythmic drug intervention.

A female baby with Apgar score of 8/1, weighing 3,280 g, with a length of 49 cm, occipitofrontal circumference of 34 cm, oxygen saturation of 95%, and a heart rate of 250 beats/min. (bpm), was immediately referred to pediatric cardiology.

On examination, the newborn had livid skin and expressed acrocyanosis, breathing rate of 58 cycles/min., blood pressure of 85/55 mmHg, liver was palpable 2 cm below the rib. The heart rate was 250 bpm, there was no murmur and peripheral pulses were palpable. Electrocardiogram (ECG) confirmed SVT with narrow QRS complex at a rate of 260 bpm (Figure 1a). Tachycardia was treated by intravenous bolus injection of adenosine-5'-triphosphate (ATP). SVT was converted to sinus rhythm after a third ATP dose of 0.3 mg/kg. No serious side effects of ATP injection occurred. However, the

Received • Примљено: February 20, 2017
Accepted • Прихваћено: March 13, 2017
Online first: March 17, 2017

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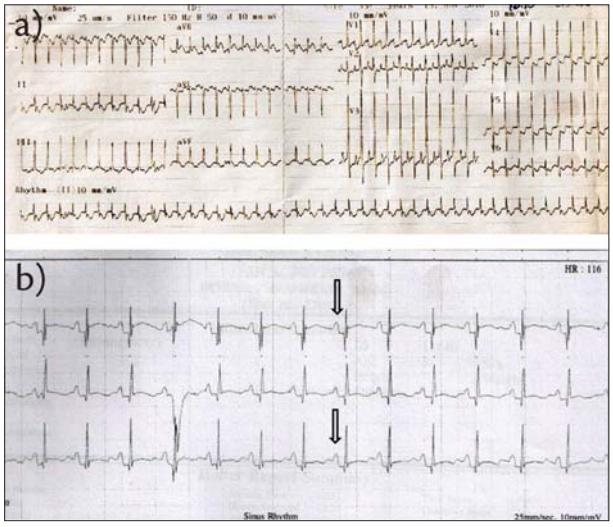

Figure 1. (a) Initial electrocardiogram showing supraventricular tachycardia; (b) electrocardiogram after converting to sinus rhythm showing giant P waves and the first degree AV block

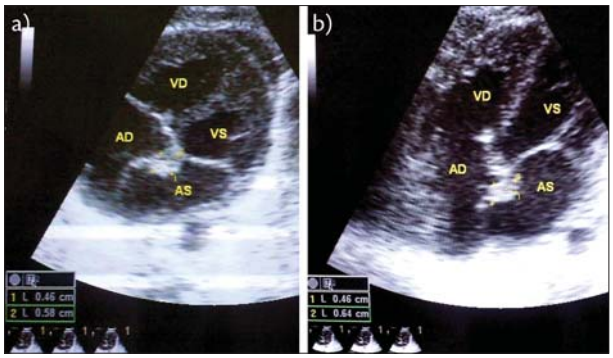

Figure 2. Neonatal echocardiogram (a) in subcostal four-chamber view and (b) long-axis four-chamber view; cardiac mass is visible in the left atrium, fixed to the primum atrial septum

rhythm frequently reverted to SVT, showing refractory supraventricular re-entrant tachycardia. During continuous ECG monitoring, when newborn was in a sinus rhythm, ECG revealed giant P waves and the first degree AV block (Figure 1b).

Echocardiography performed after the conversion to sinus rhythm showed an echogenic, well-circumscribed mass (6 × 5 mm) in the left atrium, fixed to the primum atrial septum (Figure 2). There were neither other structural defects nor signs of hemodynamic disturbance. SVT was thought to be secondary to a small cardiac tumor, especially because of its localization.

The family history did not show any birth defects, no data of benign or malignant tumors, especially no history of tuberous sclerosis. The brain ultrasonography was normal and there were no other clinical markers of tuberous sclerosis at birth.

SVT was successfully treated by a bolus of amiodarone followed by an intravenous infusion. Long-term management with oral amiodarone and propranolol had a good response.

During the one-year follow-up echocardiograms were performed every month showing regression of the cardiac mass. By the eighth month of age the tumor had completely regressed (Figure 3). Amiodarone was withdrawn

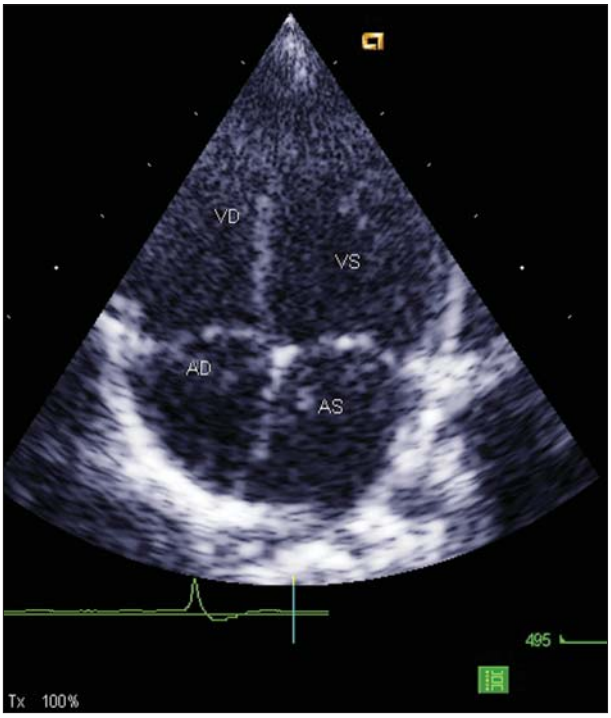

Figure 3. Echocardiogram performed in the eighth month showing that the tumor had completely regressed

at the age of six months (propranolol at eight months) and serial Holter studies documented that there had been no recurrence of tachycardia since the neonatal period.

Currently, she is a healthy six-year-old girl with no cardiac symptoms.

DISSCUSION

Primary pediatric cardiac tumors are uncommon and usually histologically benign. Rhabdomyomas are most common, followed by teratomas, myxomas, and fibromas, and the majority of them are diagnosed before the age of one year [8]. Symptoms, clinical manifestations and treatment depend on tumor size, location, number, and histological type. They can be very different and unpredictable. Thus a giant tumor in the silent zone can be asymptomatic, while a small tumor close to the conduction system, can cause arrhythmia resistant to therapy and even sudden cardiac death [2]. This report precisely describes a situation where a small tumor at a critical location caused refractory SVT that required emergency treatment and long-term management, fortunately with a good response. Our dilemma was related to the histologic type of the tumor. Rhabdomyomas appear on ultrasound as round, homogeneous, hyperechogenic mass, similar to the echocardiographic picture that we have seen in our patient. However, differential diagnosis between rhabdomyoma, fibroma, or myxoma using ultrasonography for a single cardiac mass remains very difficult [3]. Recent reports describe mostly fetuses and newborns with rhabdomyomas and associated SVT with different outcomes. The diagnosis of rhabdomyoma was confirmed either after surgical tumor removal or by autopsy [2, 4, 9]. Other authors had similar experiences

to the one we describe: tumor regressed spontaneously, and the patient had no further episodes of SVT [5].

Tumor regression and a good long-term outcome in our patient suggest that it was probably a small but unfavorably positioned rhabdomyoma, associated with fetal and perinatal SVT. Antenatal detection of fetal cardiac tumors ensures better prenatal and postnatal management [10].

In conclusion, rhabdomyomas are mostly benign and have tendency to regress, but their prognosis depends on the fact that they can be associated with life-threatening arrhythmias and tuberous sclerosis. Adequate and prompt management of arrhythmias and potential cerebral events directly affect the prognosis in these patients.

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Хиперехогено ткиво у левој преткомори и супревентрикуларна тахикардија код новорођенчета

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САЖЕТАК

Увод Примарни тумори срца код деце су ретки и већина њих се дијагностикује до краја прве године живота. Најчешће су то рабдомиоми који имају склоност ка регресији. Учесталост аритмија код ових тумора није позната и њихова појава углавном зависи од величине и локализације самог тумора.

Приказ болесника Приказујемо женско новорођенче са суправентрикуларном тахикардијом (СВТ), која је регистрована пренатално. Порођај је завршен хитним царским резом, при чему није урађена фетална ехокардиографија, нити је покушана медикаментна конверзија тахикардије. Електрокардиограм је потврдио тахикардију (260/мин.) са уским *QRS* комплексом која је конвертована у синусни ритам након треће интравенске болус дозе аденозин-5′-трифосфата. Синусни ритам се тешко одржавао због упорне СВТ. На ехокардиографском прегледу, после конверзије у синусни ритам, виђено је хиперехогено, добро ограничено ткиво у левој преткомори, фиксирано за дистални део преткоморске преграде. Није било других морфолошких промена. СВТ је успешно је заустављена болусом, а затим интравенском инфузијом амиодарона. Тахикардија је надаље успешно контролисана перорално комбинацијом амиодаронаи и бета блокатора. Током једногодишњег ехокардиографског праћења, које је спровођено једном месечно, регистрована је постепена и потпуна регресија тумора. Тахикардија се није више поновила и девојчици је укинута терапија.

Закључак Регресија тумора и добра дугорочна прогноза код нашег болесника указују на то да се вероватно радило о малом, али „лоше постављеном" рабдомиому, који је узроковао СВТ код фетуса и након рођења код новорођенчета. Прогноза и исход болести код оваквих болесника директно зависе од правовремене дијагнозе и брзог и адекватног лечења тумором узрокованих аритмија опасних по живот.

Кључне речи: тумори срца; суправентрикуларна тахикардија; рабдомиом; новорођенче

DOI: https://doi.org/10.2298/SARH160906069S
UDC: 617.721-003.4-089

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Recurrence of a small primary iris stromal cyst following treatment with Nd:YAG laser photodisruption in an adult

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SUMMARY
Introduction Primary acquired iris stromal cyst is rare in adults. In this group, they are generally stable lesions which require no treatment.
Case outline We describe a rare case of a small primary iris cyst in a 39-year-old patient, associated with unusual signs of irritation. Ultrasound biomicroscopy demonstrated iris stromal cyst measuring 3 × 2 mm. A neodymium-doped yttrium aluminium garnet (Nd:YAG) laser cystotomy was engaged as the least invasive treatment approach. However, the cyst recurred soon after repeated laser treatment and sector iridectomy with excision of the cyst was performed. Five years after surgery there was no evidence of recurrence.
Conclusion Although more benign clinical course of primary stromal iris cyst is generally assumed in adults as compared to children, complete cyst removal seems to be mandatory for preventing cyst recurrence regardless of the cyst size or patient age. To the authors' knowledge this is the first documented report of Nd:YAG laser photodisruption of acquired primary iris stromal cyst in an adult.
Keywords: primary iris stromal cyst; laser cystotomy; treatment

INTRODUCTION

Primary iris stromal cyst is uncommon unilateral lesion, usually presenting in the first few years of life [1]. Acquired primary iris stromal cysts in adults are extremely rare [2]. Most of these lesions behave in a benign fashion. Only a few cases in which rapid growth of iris stromal cyst required treatment have been described in adults.

Here we present a rare case of a small iris stromal cyst in an adult, associated with unusual symptoms. The aim is to present our experience in treating this kind of cyst and to determine the outcome of minimally invasive treatment approach using Nd:YAG laser.

CASE REPORT

Here we present a case of a 39-year-old man with a six-month history of tearing and redness of the left eye. There was no history of trauma or previous intraocular surgery. Visual acuity was 20/20 in both eyes, but the patient nevertheless complained about strong irritation associated with epiphora and photophobia, which interfered with his daily and particularly working activities. Intraocular pressure was within normal range. Slit-lamp examination of the left eye revealed a transparent inferior midzonal iris cyst (Figure 1). The cyst wall was opposite the corneal endothelium; however, there was no corneal edema. The right eye and the remaining clinical examination were normal. Ultrasound biomicroscopy confirmed the presence of a midzonal stromal iris cyst measuring 3 × 2 mm with corneal touch (1.78 mm). There was no secondary angle closure. The posterior iris epithelium was intact (Figure 2).

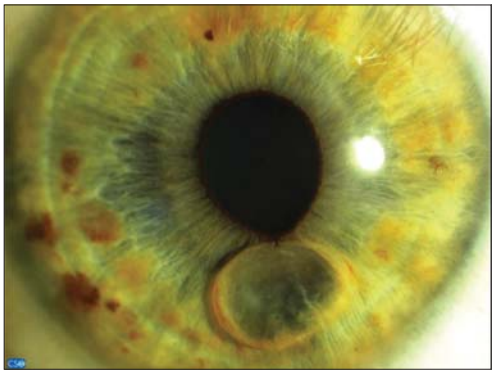

Figure 1. Clinical photograph of a small primary acquired iris stromal cyst at presentation

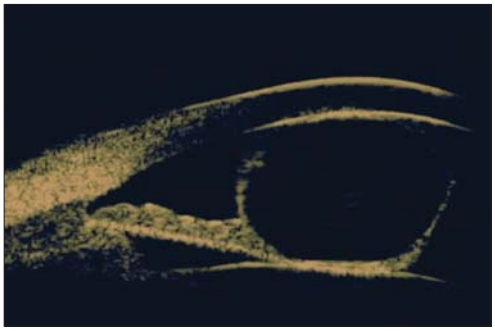

Figure 2. Ultrasound biomicroscopy showing the cyst extending into iris stroma

Received • Примљено: September 6, 2016
Revised • Ревизија: November 16, 2016
Accepted • Прихваћено: December 22, 2016
Online first: March 14, 2017

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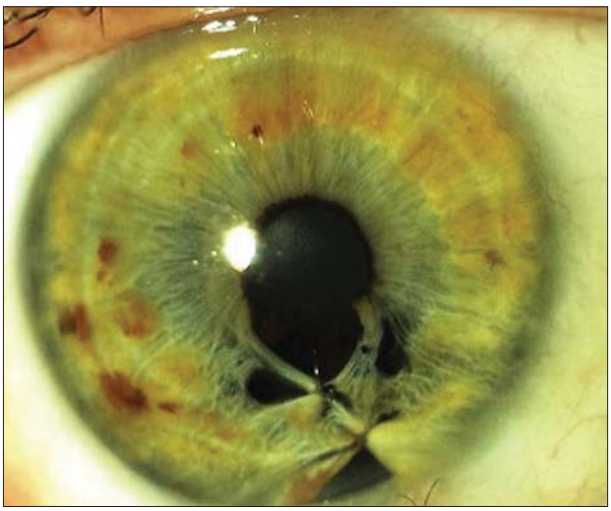

Figure 3. Clinical photograph showing the eye after surgical excision of the cyst

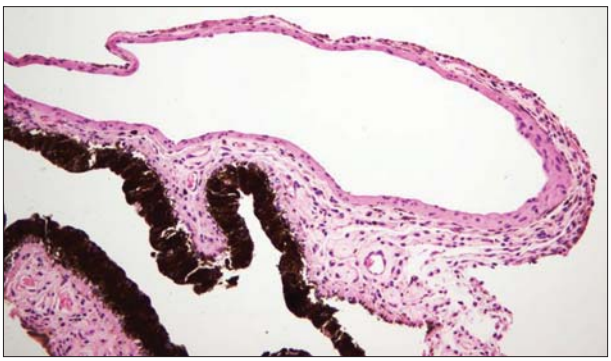

Figure 4. Histopathology of excised tissue demonstrating intrastromal iris cyst (H&E, ×200)

Short course of steroid drops, 0.1% dexamethasone four times a day for four weeks, was ineffective in reducing signs and symptoms of irritation. Considering slow progressive growth of the iris stromal cyst, long-term consequences of steadily enlarging corneal endothelial touch surface as well as significant irritation of the left eye affecting the quality of life of this patient, we decided to perform laser photodisruption with cyst deflotion, as the first step of the procedure. Nd:YAG laser puncturing of the cyst wall was done at a setting of 1.9 mJ for a total of five spots. The cyst collapsed and fluid leaked out in the anterior chamber. Postoperative medication included topical steroids and antibiotics four times a day for two weeks. However, at three weeks of follow-up, the cyst reccured and more extensive Nd:YAG laser photodisruption of the entire anterior cyst wall was carried out.

One month later, the cyst again recurred and surgical excision was performed. Intraoperatively, a limbal incision was made extending slightly longer than the diameter of the cyst. The anterior cyst wall was separated from the corneal endothelium by gentle viscoelastic dissection. Iris resection, inculding entire cystic lesion and iridoplasty, was performed (Figure 3). Postoperative uncorrected visual acuity was 20/20 and intraocular pressure measured 18 mmHg.

Histopathology showed the intrastromal (not epistromal) iris cyst lined by a nonkeratinizing stratified epithelium (two to three layers of cubic epithelial cells), a cellular layer resembling conjunctival or corneal epithelium. No intraepithelial goblet cells were found. Superficial iridial stroma was streched/rarefied. No superficial or deep lymphoplasmocytic infiltrate was found arround the cyst. Posterior iris epithelial layers were intact (Figure 4). Five years after surgery, the clinical findings remained stable and there was no evidence of recurrence.

DISCUSSION

Primary iris stromal cyst is uncommon [1, 3]. Recently, Shields et al. [4] presented data on 3,680 iris tumors managed over a 36-year period. In this study, only 3% of cases were cystic tumors originated from iris stroma. Of the 96 iris stromal cysts, congenital iris stromal cyst was most common in children (71%, 25/35 cases) and acquired stromal cyst was most common in senior adults (19/49 cases, 39%) [4]. Primary iris stromal cysts are rarely seen in adults [2]. These kinds of cysts are larger in children (mean size of 7.8 mm) than in teenagers and adults (mean size of 4.7 mm). Overall, associated findings are more common in children than in the letter group requiring treatment in the majority of cases (88%). On the other hand, primary iris stromal cysts in adolescents and adults necessitated intervention in only two cases (25%) [2].

Here, we presented an unusual case of a relatively small primary acquired iris stromal cyst (the largest base of the cyst was 3 mm), in an adult patient, associated with signs of irritation, epiphora, and a slow progressive enlargement. Management strategy of such iris cysts is not clearly defined. Treatment approach is generally based on the anticipated cyst complications to the eye and its effect on visual acuity [5]. Different treatment approaches have been advocated, including excision, needle aspiration, cryotherapy, injection of chemical substances, treatment with argon laser, and a combination of these modalities. However, recurrences are common, mainly due to incomplete cyst excision.

Interestingly, Nd:YAG laser cystotomy, as a simple and effective treatment, has been reported in two children [6]. In these cases the cyst wall collapsed without complications and remained free of recurrences during the one year follow-up period. In iris stromal cysts diagnosed later in life, more favorable prognosis has been observed. Spontaneous collapse of this kind of cysts has also been described [7]. Thus, owing to persistent irritation, we decided to use Nd:YAG laser for puncturing the cyst wall, as the least invasive treatment approach, for a small primary iris stromal cyst. However, due to the cyst recurrence three weeks afterwards, an extensive Nd:YAG laser photodisruption of the entire anterior cyst wall was carried out. Although less aggressive clinical course of stromal iris cyst is generally assumed in adults as compared to children, in our patient the cyst recurred soon after repeated laser treatment.

We believe that residual cyst epithelium overlying the iris surface was the source of an early cyst recurrence in our patient. This was also addressed in previous documented

recurrences in children [8, 9]. Therefore, surgical excision was performed and there was no complication or cyst recurrence during the five-year follow-up period.

In conclusion, contrary to the expected outcome, our conservative stepwise approach using Nd:YAG laser for a small primary iris stromal cyst in adulthood was unsuccessful. In the view of here presented case as well as previous reports [10], it seems important to provide adequate treatment while the cyst is still small, since the recurrence rate is likely to be only a function of the adequacy of initial epithelial cyst removal regardless of the cyst size or patient age.

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Рецидив мале примарне стромалне цисте дужице код одрасле особе након фотодисрупције ласером *Nd:YAG*

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САЖЕТАК

Увод Примарно стечена стромална циста дужице веома је ретка код одраслих особа. У овом добу то су стационарне лезије које не захтевају лечење.

Приказ болесника Описали смо један редак случај мале примарне цисте дужице праћене неуобичајеним знацима иритације код мушкарца старог 39 година. Ултразвучном биомикроскопијом приказана је стромална циста дужице величине 3 × 2 *mm*. Помоћу ласера *neodymium-doped yttrium aluminium garnet* (*Nd:YAG*) урађена је цистотомија, као најмање инвазиван терапијски приступ. Међутим, циста је рецидивирала у кратком временском периоду и после поновљене ласерске интервенције, па је урађена ексцизија цисте са секторастом иридектомијом. Пет година после хируршке интервенције није било знакова рецидива цисте.

Закључак Мада се сматра да примарна стромална циста дужице има бенигнији клинички ток код одраслих особа у поређењу са децом, укључањање цисте у целини неопходно је у циљу превенције рецидива без обзира на њену величину или старост болесника. Према нашем сазнању, ово је први документовани приказ фотодисрупције ласером *Nd:YAG* примарно стечене стромалне цисте дужице код одрасле особе.

Кључне речи: примарна стромална циста дужице; ласерска цистотомија; терапија

DOI: https://doi.org/10.2298/SARH160712061C
UDC: 616.233-006

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Mediastinal lymphangioma in an adult with a tracheal bronchus

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SUMMARY

Introduction Lymphangiomas, also known as cystic hygromas or cystic lymphangiomas, are cystic abnormalities of the lymph vessels and they are rare benign tumors. Tracheal bronchus (*Bronchus suis* or "pig bronchus") is a very rare congenital anomaly.
The aim of this work is to present a very rare case of a lymphangioma with tracheal bronchus.

Case outline The article presents the rare case of a 35-year-old otherwise healthy man, who was admitted to our thoracic surgery department with a mediastinal tumor. On performing bronchoscopy a tracheal bronchus was found. A thoracic CT scan revealed a well-circumscribed mass in the superior and anterior mediastinum measuring 37 x 39 x 59 mm. First a Carlens mediastinoscopy, and then a right parasternal Chamberlain mediastinotomy were performed. The final pathological diagnosis of lymphangioma was made. In this case, surgery was not performed because the patient was asymptomatic and the tumor did not grow larger during follow-up.

Conclusion The lymphangioma of the mediastinum in an adult is a rare and benign condition with a good prognosis, but it should be considered in a differential diagnosis of mediastinal tumors. We recommend only a minimally invasive diagnostic approach (parasternal mediastinotomy) when the patient is asymptomatic.

Keywords: mediastinum, lymphangioma; tracheal bronchus; mediastinoscopy; parasternal mediastinotomy

INTRODUCTION

Lymphangiomas, also known as cystic hygromas or cystic lymphangiomas, are cystic abnormalities of the lymph vessels [1, 2]. They are rare benign tumors [1]. Nearly 90% of them occur in children up to the age of two years [3]. They appear mainly in the neck (75%) and axillary regions (20%) [4]. Other locations of lymphangiomas are mesenteric, retroperitoneal, orbital, pancreatic, and mediastinal (1%) [5]. They are predominantly congenital or sometimes acquired due to chronic lymphatic obstruction (e.g. infection, radiation). A malignant transformation of a lymphangioma has not been observed. Approximately 200 cases of lymphangiomas in adults have so far been described in literature [6].

Tracheal bronchus (*Bronchus suis* or "pig bronchus," and "tracheal diverticulum" as a variant) is a very rare congenital anomaly with a frequency of about 0.5% of pediatric bronchoscopy procedures [7].

The aim of this work is to present a very rare case of lymphangioma with tracheal bronchus.

CASE REPORT

A 35-year-old otherwise healthy man (A.S.), a cigarette smoker (15 packs per year) was admitted to our thoracic surgery department with symptoms of chronic fatigue and elevated (subfebrile) body temperature, which had lasted for three weeks. His past medical history was unremarkable, except for pulmonary tuberculosis in his adolescence and arterial hypertension. On admission the patient was in good general health. Physical examination revealed normal findings. Laboratory studies showed normal blood morphology and urinalysis. On performing bronchoscopy, a tracheal bronchus (a congenital anomaly) was found, starting from the right lateral wall of the trachea at a distance of less than 2 cm from the carina. A thoracic computed tomography (CT) scan revealed a well-circumscribed mass located in the superior and anterior mediastinum, measuring 37 × 39 × 59 mm in cross section (Figure 1), and displacing the superior vena cava and the right brachiocephalic vein. On October 30, 2012, a Carlens mediastinoscopy

Received • Примљено: July 12, 2016
Revised • Ревизија: November 3, 2016
Accepted • Прихваћено: November 16, 2016
Online first: March 10, 2017

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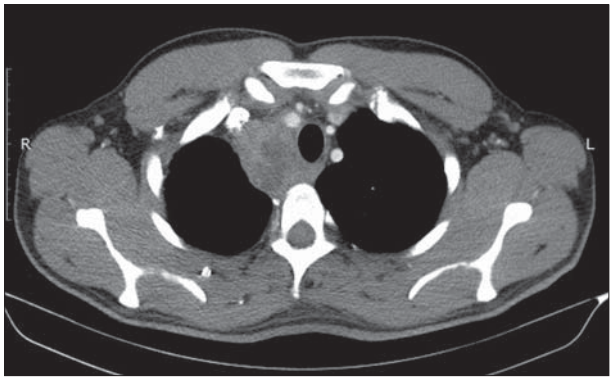

Figure 1. Contrast-enhanced axial CT scan of the thorax showing a well-circumscribed mass located in the superior and anterior mediastinum, measuring 37 × 39 × 59 mm, encasing the superior vena cava and the right brachiocephalic vein

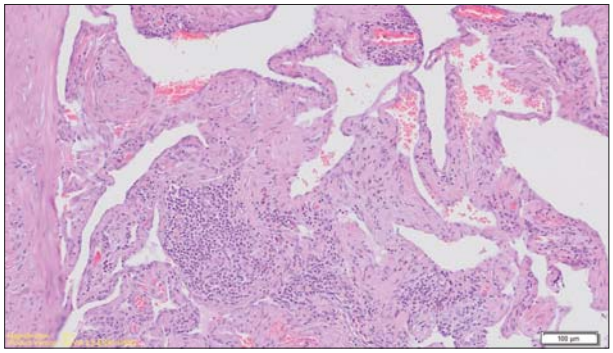

Figure 2. Lymphangioma; there are numerous, irregular vessels composed of endothelial and smooth muscle cells with lymphoid tissue between the vessels; erythrocytes within the lumen of some vessels might be misleading (H&E, ×70)

was performed under general anesthesia. The mediastinal tumor was biopsied with forceps and straw-colored serous fluid of 50 ml in volume was aspirated. The postoperative recovery was uneventful. The pathological examination revealed only necrotic masses, deposits of hemosiderin as well as non-specific fibrotic and/or inflammatory granulation of the tissue. The histopathology report stated that the finding was an organizing hematoma. The acid-fast bacteria culture of the sample was negative. A positron emission tomography scan was performed and confirmed the presence of the soft-tissue tumor in the mediastinum (maximum standardized uptake value of 4.7), which was characteristic of a low-grade proliferation process. Therefore, the decision to perform a Chamberlain parasternal mediastinotomy was taken, and this was carried out on February 14, 2013. During this procedure the right second costal cartilage was removed and the tumor of the anterior mediastinum was sampled. Postoperative recovery was uneventful. The patient was discharged on the first postoperative day. Histopathological examination revealed lymphangioma. H&E stain showed numerous, irregular vessels composed of endothelial and smooth muscle cells with lymphoid tissue between the vessels (Figure 2). Immunohistochemistry showed a positive reaction with the monoclonal antibody D2-40 for podoplanin (as a marker for the presence of a lymphatic endothelium), CD31 and

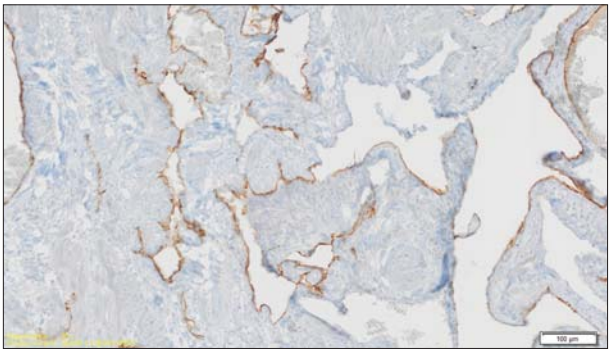

Figure 3. Positive immunohistochemical staining (brown color) with the D2-40 monoclonal antibody recognizing podoplanin, which is the membrane mucoprotein of lymphatic endothelium (×70)

CD34 (indicating the endothelial origin of tissue) (Figure 3). But the reaction to pancytokeratin AE1/AE3 was negative. There were no changes in the patient's condition in follow-up visits.

DISCUSSION

Lymphangiomas are benign tumors of mesodermal origin [8, 9]. They are divided into the following types: cystic (predominant in the thorax), cavernous, or mixed type [1, 10]. Thoracic lymphangiomas remain asymptomatic for many years as they grow slowly, and they are usually discovered incidentally. In this case, the patient was presented with only mild symptoms (chronic fatigue and slightly elevated body temperature), which soon subsided. Only large tumors may cause symptoms by compressing the adjacent anatomical structures, i.e. the tracheo-bronchial tree, the esophagus, or the superior vena cava [9]. Lymphangiomas are found more often in the superior and anterior mediastinum [2, 11, 12]. They account for 0.7–4.5% of all mediastinal masses [11]. The differential diagnosis includes a bronchogenic cyst, a pericardial cyst, a cystic thymoma, a cystic teratoma, a lymphoma, a goiter, a hematoma, or an aneurysm of the brachiocephalic trunk [5, 10, 11, 12]. Thoracic CT scan is helpful in determining the extent of the disease. The CT feature of lymphangioma is a cystic lesion with well-defined margins and without calcifications, which envelopes the thoracic structures [10, 12]. A histological examination with immunocytochemical staining for CD31 confirms the final diagnosis [11]. Some authors recommend complete surgical excision, usually via the right lateral thoracotomy [2, 4, 5, 6, 8, 10]. Others, for example Conte et al. [3], or Gorska et al. [1], advocate a conservative approach (watchful waiting) if the patient is asymptomatic. In this case, the non-invasive option was chosen as upfront surgery is not sufficiently evidence-based in literature. Lymphangioma of the mediastinum in an adult is a rare and benign condition with a good prognosis. It should be considered in a differential diagnosis of mediastinal tumors. We recommend only a minimally invasive diagnostic approach (parasternal mediastinotomy) when the patient is asymptomatic.

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Лимфангиом медијастинума код одраслог болесника удружен са трахеалним бронхом

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САЖЕТАК

Увод Лимфангиоми, познати и као цистични лимфангиоми или хигроми, јесу аномалије лимфних судова класификоване и као бенигни тумори. Трахеални бронх је врло ретка урођена аномалија.

Циљ овог рада је приказ врло ретког случаја лимфангиома са трахеалним бронхом.

Приказ болесника Приказан је мушкарац, 35 година стар, иначе здрав, примљен на одељење грудне хирургије због медијастиналног тумора. На бронхоскопији је нађен трахеални бронх. Компјутеризована томографија груди указала је на масу димензија 37 × 39 × 59 *mm* у предњем горњем медијастинуму. Урађена је прво Карленсова медијастиноскопија, а затим и Чемберленова десна парастернална медиастинотомија, а патохистолошки налаз потврдио је дијагнозу лимфангиома. Хируршко лечење није предузето јер у посматраном периоду медијастинална маса није расла, а болесник је био без симптома.

Закључак Лимфангиоми медијастинума код одраслих су ретки и доброћудни са добром прогнозом, али је неопходна јаснија диференцијална дијагноза од других медијастиналних тумора. Код асимптоматских болесника препоручујемо минимално инвазивни дијагностичи приступ (парастернална медијастинотомија).

Кључне речи: медијастинум, лимфангиом; трахеални бронх; медијастиноскопија; парастернална медијастинотомија

DOI: 10.2298/SARH160630058B
UDC: 616.33-089-053.2

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Simultaneous combined laparoscopic-endoscopic removal of a large gastric trichobezoar and gastric polypectomy

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SUMMARY

Introduction Trichobezoars and gastric polyps are very rare conditions in children and may pose a diagnostic and therapeutic challenge. The purpose of this work is to present our successful experience using combined laparoscopic-endoscopic procedure for simultaneous treatment of a trichobezoar and gastric polyp in the same patient.

Case outline We present an unusual case of a 15-year-old girl whose symptoms included abdominal pain, non-bilious vomiting after feeding, including undigested food and sometimes hair. Positive history of trichophagia indicated that a trichobezoar could be the reason for her problems. Endoscopy and ultrasound examination revealed a trichobezoar occupying almost the entire capacity of the stomach, as well as one oval polyp in the prepyloric area of the antrum. Simultaneous combined laparoscopic-endoscopic rendezvous procedure was performed. The trichobezoar (14 × 6 cm) and the gastric polyp (2.2 × 1.7 cm) were completely removed laparoscopically through anterior gastrotomy, with great support of an adequate endobag and mechanical fragmentation of trichobezoar. The postoperative course was uneventful.

Conclusion This case shows that diagnostic endoscopy is valuable and that the combined laparoscopic-endoscopic technique is feasible, safe and recommended treatment for simultaneous removal of a gastric trichobezoar and gastric polypectomy.

Keywords: trichobezoar; gastric polyp; laparoscopy; endoscopy

INTRODUCTION

The purpose of this work is to present our positive experience using combined laparoscopic-endoscopic procedure and to review current literature in resolving joint appearance of trichobezoars and gastric polyps. A formation of undigested material in the gastrointestinal tract is bezoar [1]. In a situation of a long-term ingestion of hair, i.e. trichophagia, this type of bezoar is called trichobezoar, which is at the same time the most common form of bezoar [2]. Hair cannot be digested and due to its smooth nature also cannot be propulsed by peristalsis, which causes formation of a bezoar within the stomach over a certain period. The hair in a trichobezoar always appears black because of the influence of gastric acid on hair proteins [1, 3]. In literature, the association between bezoars and gastric polyps is relatively frequently described; however, it is not so widely appreciated. Even though biopsies of gastric polyps usually show an inflammatory origin, in some cases their malignant alteration was found [4]. A multidisciplinary approach with early diagnosis and surgical removal of gastric bezoars and polyps are essential [1, 3, 5].

CASE REPORT

Our case was a 15-year-old girl with abdominal pain and vomiting after feeding. The vomit included undigested food and sometimes hair. Due to occasional gastrointestinal bleeding she appeared extremely pale. Her abdomen was non-distended without palpable mass. Positive history of trichotillomania and trichophagia led us to believe that trichobezoar could be the reason for her problems. Her mother noticed that she had lost weight. Laboratory values did not show any abnormalities, especially serum proteins and particularly albumins, amylase, and lipase levels, and reactants of acute phase of inflammation such as C-reactive protein and erythrocyte sedimentation rate. At endoscopy and ultrasound examination a trichobezoar occupying almost entire capacity of the stomach was noted. Furthermore, one oval sessile polyp, not bleeding actively, was revealed in the prepyloric area of the antrum.

Combined laparoscopic-endoscopic rendezvous procedure was performed. The role of endoscopy during this "rendezvous" procedure was to enable precise placement of a gas-

Received • Примљено: June 30, 2016
Revised • Ревизија: September 6, 2016
Accepted • Прихваћено: October 4, 2016
Online first: March 7, 2017

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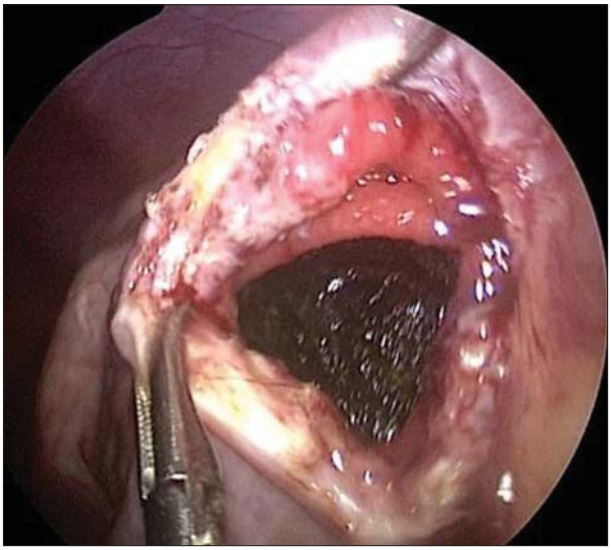

Figure 1. Trichobezoar seen through created anterior gastrotomy

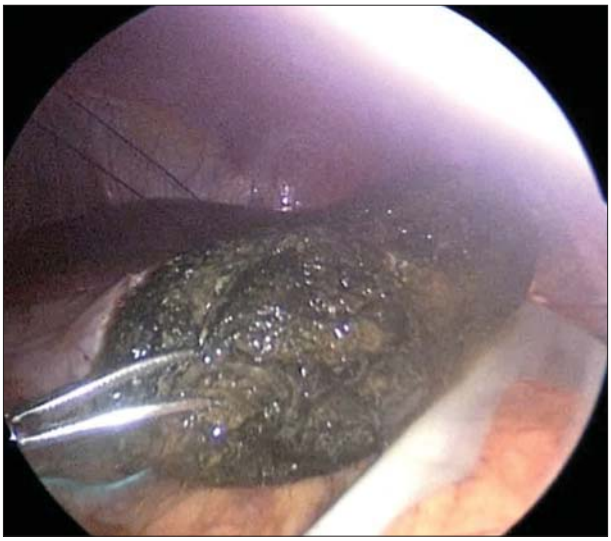

Figure 2. Removal of the trichobezoar (14 × 6 cm) from the stomach using a specially modeled endobag (Endo Catch™ 15 × 10 cm)

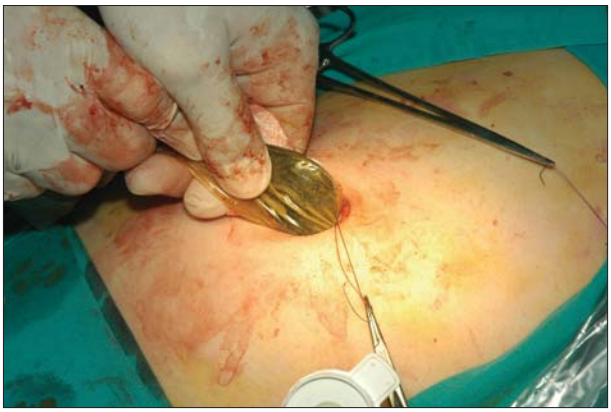

Figure 3. Removal of the trichobezoar from the abdominal cavity through the enlarged umbilical port

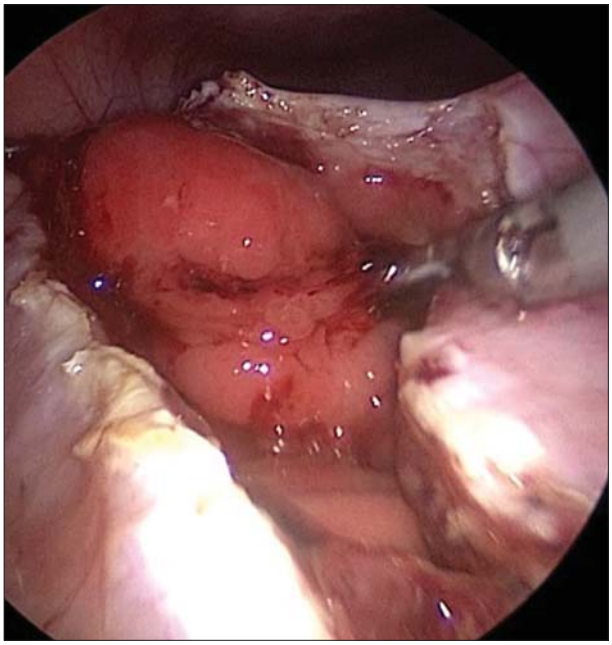

Figure 4. Gastric polypectomy (2.2 × 1.7 cm)

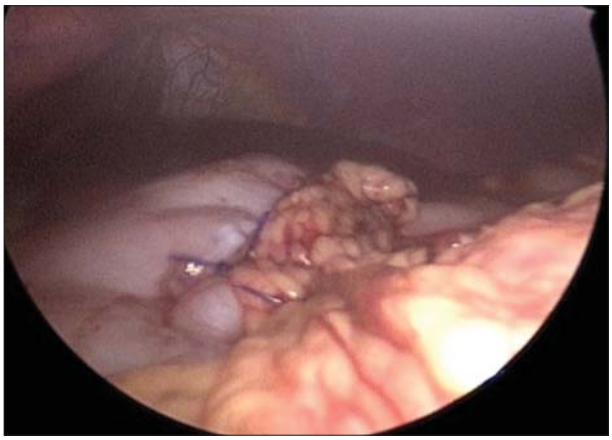

Figure 5. Omentoplasty using non-absorbable 3-0 suture

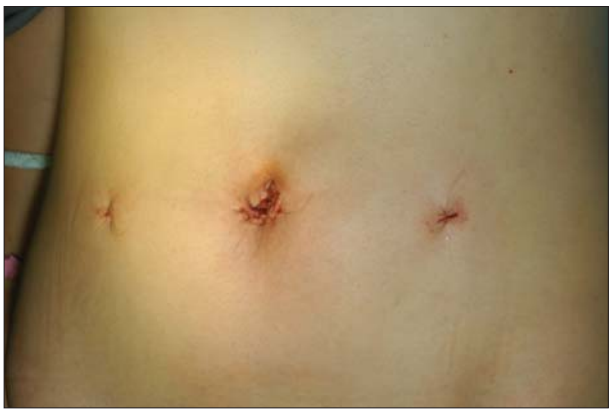

Figure 6. The aesthetic result of the operation

trotomy incision, to exclude the possible existence of the Rapunzel syndrome, and to navigate surgeon to find the gastric polyp. The most important part of endoscopy during the above mentioned procedure is to control bleeding. After endoscopic navigation, laparoscopic exploration of the entire intestine was the first part of the operation. The trichobezoar (14 × 6 cm) was completely removed laparoscopically through created anterior gastrotomy (Figure 1), also using specially modeled endobag (Endo Catch™ 15 × 10 cm; Covidien Ltd., Dublin, Ireland) (Figure 2). Incision of the first umbilical port was minimally enlarged, and before complete extraction of the trichobezoar from the

abdomen, its mechanical fragmentation in the endobag was performed without spilling into the abdominal cavity (Figure 3). The trichobezoar had a highly offensive smell, which was the reason for smearing, culturing and administering antibiotics postoperatively. Additionally, gastric polypectomy (dimensions 2.2 × 1.7 cm) was also done laparoscopically using the Ultracision® Harmonic® (Ethicon Endo-Surgery Inc., Cincinnati, OH, USA) scalpel and gastrotomy through the same (Figure 4). After resection and exact hemostasis, the polyp was removed from the abdominal cavity by placing an endoscopic extraction basket (Roth Net®; US Endoscopy, Mentor, OH, USA) through one of the created ports. Histopathological examination confirmed it to be a hyperplastic gastric polyp.

Firstly, mucosal defect after polypectomy was sutured using absorbable sutures 4-0; afterwards, the gastric wall was closed by using intracorporeal suturing. Omentoplasty completed the procedure (Figure 5). For that we used non-absorbable 3-0 interrupted suture extramucosally. The same stiches were applied for omentoplasty. Finally, the nasogastric tube was placed. The endoscopist controlled the intraluminal hemostasis throughout the procedure. The postoperative course was uneventful, per oral nutrition started after three days (Figure 6). The gastroenterologist and psychiatrist continued the treatment.

DISCUSSION

Trichobezoars ("hair ball") are usually located in the stomach. Incidentally, a gastric trichobezoar can extend through the pylorus into distal parts of the gastrointestinal tract. This rare type is called Rapunzel syndrome. Between many complications of this condition, the most frequent are mucosal erosion, ulceration or even perforation of the stomach and intestine, intestinal invagination, jaundice, pancreatitis, and extremely rarely even death [1].

In child population, trichobezoars are found in 90% of the cases. On the other hand, they are an uncommon condition of which only approximately 300 cases have been reported in papers [2]. Main causes are habitual hair pulling called trichotillomania and ingestion of hair called trichophagia, which are usually related to obsessive-compulsive disorders and/or depression [2]. In our case, the child was not controlled preoperatively by a psychiatrist. Physicians should always keep in mind the possibility of a bezoar formation especially after gastric surgery, in case of celiac disease, diabetes mellitus, myotonic muscular dystrophy, cimetidine therapy, etc. [1, 2, 6]. In our case, positive history of trichophagia and visible weight loss pointed us to the right direction in diagnosis and appropriate treatment.

Standard clinical findings are usually non-specific. In literature, Lamerton's sign, a large mobile epigastric mass, is described as a typical clinical manifestation. It is clear that it may pose a diagnostic challenge when during clinical examination it is not found [1].

Ripolles et al. [7] compared conventional abdominal radiographs, sonography, and computed tomography. A typical computed tomography image shows a well-defined intraluminal ovoid heterogeneous mass with interspersed gas [2, 4]. However, endoscopy is essential and the best choice in the diagnosis process [8]. In our case, sonography and endoscopy revealed huge trichobezoar and polypoid mass in the antrum of the stomach.

The endoscopist should closely monitor if the pylorus is normal and that there is no distal obstruction. It is well-known that in 17% of cases trichobezoar can be multiple. Also, distal migration of the subsidiary fragments may cause complete or incomplete small bowel obstruction [8]. This is the reason why the entire digestive tract should be examined thoroughly to prevent secondary ileus [8]. We also started the operation with endoscopic navigation and laparoscopic exploration of the entire intestine.

Current data propose many therapy solutions such as endoscopy, standard laparotomy, minimal invasive surgery, and even ineffective medical treatment with enzymatic degradation [1, 2, 9, 10].

Many authors suggest standard open surgery as appropriate treatment for children with trichobezoars, because of better results of this method in comparison with endoscopy and laparoscopy [1, 11]. Occasional reports inform about new therapeutical methods like extracorporeal shock wave lithotripsy, laser, and the combination of endoscopy and laparoscopy. The last report from China even claimed 100% success rate in resolving trichobezoars using a new "explosive" technique through the endoscope [6]. However, clinical experience is still modest for their promotion or suggestion.

Except removal by conventional laparotomy, in some papers, a minimal invasive approach, such as laparoscopy is also proposed. Nirasawa et al. [12] reported successful laparoscopic removal of a trichobezoar for the first time. After this presentation only six other similar reports were published [10]. Still, progression in the laparoscopic technique, in general and peculiarly for the removal of trichobezoars, and exploration of the entire intestine, is not so easy to achieve [13, 14].

Despite the fact that literature describes combined laparoscopy and endoscopy in the management of trichobezoars, there is only one publication addressing this combined technique for the same pathology. [14] We have used this technique at our institute since 2009.

A particularly limiting factor for complete laparoscopic or combined laparo-endoscopic procedure is the size of a trichobezoar. In our case, the largest available endobag measuring 15 × 10 cm allowed for laparoscopic removal of a trichobezoar. However, to prevent abdominal contamination it was necessary to gently pull trichobezoar from the stomach into the endobag. Also, the only way to insert the trichobezoar into the endobag was to pull it down and place it in its width. In the literature, minimal and maximal dimensions of gastric bezoars are 6 × 6 × 5 cm and 15 × 10 × 10 cm, respectively, and for intestinal bezoars the measurements are 3 × 3 × 4 cm to 4 × 7 × 7 cm [8]. Specified sizes of trichobezoars allow their placement in standardized and commercially available endobags. This is also important because laparoscopic approach in trichobezoar treatment may seem less attractive due to

possible spilling of contaminated hair into the abdominal cavity [9]. As it is well-known that wound infection even after conventional laparotomy is a frequent complication, bacteriological analysis of a trichobezoar is mandatory [8, 9]. Postoperative administration of antibiotics is obligatory [9]. Trichobezoar recurrence is an additional complication and can occur if the underlying psychological condition is not treated [6, 8].

Bates et al. claimed that formation of gastric polyps is a result of chronic irritation of the gastric mucosa by bezoars [5]. In our case, histopathological analysis confirmed the hyperplastic characteristic of the extirpated polyp.

Our first successful result using combined laparoscopic-endoscopic procedure gave us hope that combining minimal invasiveness with optimal efficiency provides a safe and efficient therapy. Only one similar therapeutical approach can be found in current literature, where trichobezoar fragmentation was made laparoscopically while endoscopy was used for removal of the fragments [13, 14]. In any case where there is information about trichotillomania and trichophagia it is advisable to perform an endoscopic examination.

A combined laparoscopic-endoscopic technique is a feasible and recommended treatment for simultaneous removal of a gastric trichobezoar and gastric polypectomy.

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Истовремено лапароскопско и ендоскопско уклањање гастричног трихобезоара и гастричног полипа

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САЖЕТАК

Увод Трихобезоар и гастрични полип су веома ретки у дечјем узрасту и могу представљати дијагностички и терапијски изазов.

Циљ овог рада је да се прикаже успешно искуство у примени комбиноване лапаро-ендоскопске процедуре приликом истовременог решавања трихобезоара и гастричног полипа код детета.

Приказ болесника Приказујемо 15-годишњу девојчицу са симптомима бола у трбуху, повраћања желудачног садржаја са примесама несварене хране и длака. Анамнестички податак о трихофагији указује на постојање трихобезоара као могућег узрока постојећих тегоба. Ендоскопски и ултразвучни налаз потврдили су дијагнозу. Трихобезоар заузима скоро у потпуности лумен желуца, а у препилоричној регији верификује се постојање полипа. Лапароскопско-ендоскопском, тзв. *rendez-vous* процедуром, трихобезоар димензија 14 × 6 *cm* и гастрични полип димензија 2,2 × 1,7 *cm* су одстрањени кроз предњу гастротомију. Примењен је ендобег, а претходно је трихобезоар фрагментован. Постоперативни период је протекао уредно.

Закључак Овај приказ нам показује значајне могућности комбиноване лапароскопско-ендоскопске технике као сигурног и препорученог терапијског начина у истовременом уклањању гастричног трихобезоара и гастричног полипа.

Кључне речи: трихобезоар; полип; лапароскопија; ендоскопија

DOI: 10.2298/SARH160907070M
UDC: 616.61-085-053.3

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Congenital nephrotic syndrome may respond to cyclosporine A – A case report and review of literature

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SUMMARY

Introduction Congenital nephrotic syndrome (CNF) is manifested at birth or within the first three months of life. The Finnish-type of CNF is caused by the mutation of the NPHS1 gene, which encodes nephrin in the podocyte slit diaphragm. It is a very severe disease, for which immunosuppressive therapy is not advised. Here we describe a patient with CNF who responded to CsA by partial remission.

Case outline A girl aged 2.5 months presented with severe non-syndromic steroid-resistant nephrotic syndrome. She needed aggressive support including daily albumin infusions and diuretics. Substitution of vitamin D, thyroxin, and anticoagulants were regularly administered. She was also treated with angiotensin converting enzyme inhibitor, without clear benefits regarding proteinuria. In addition, she received intravenous gamma-globulin replacement therapy and antibiotics during frequent infections. While waiting for the results of genetic analyses and faced with many problems related to daily albumin infusions, infections, and thromboembolic complications, cyclosporine A (CsA) was introduced as an alternative to early nephrectomy and consequent renal failure. The patient responded by partial remission and CsA treatment continued at home without the albumin infusions. After almost five years since the beginning of the treatment, the patient's renal function remains unreduced.

Conclusion Our case demonstrates that CsA can induce partial remission in patients with genetic forms of steroid-resistant nephrotic syndrome without influencing the glomerular filtration rate. However, its long-term effect and safety should carefully be monitored.

Keywords: NPHS1 gene mutation; nephrin; steroid resistant nephrotic syndrome; children

INTRODUCTION

Congenital nephrotic syndrome (CNS) is manifested at birth, or within the first three months of life. It is mostly an inherited disease caused by an autosomal recessive mutation in the NPHS1 gene, which encodes a transmembrane protein designated as nephrin [1]. Nephrin belongs to immunoglobulin family of cell adhesion molecules. It is a structural protein of the glomerular podocytes slit diaphragm that interacts with two other podocytes proteins – podocin and CD2AP. This explains why nephrin abnormalities cause severe proteinuria [1].

Being the most common in Finland, with an incidence of 1.2 per 10,000 live births, CNS due to the NPHS1 gene mutation is also known as Finnish nephrotic syndrome (CNF) [2]. The causative abnormal gene has been localized to the long arm of chromosome 19 in both Finnish and non-Finnish families [3, 4]. CNF is a very severe disease with prenatal increased proteinuria, premature birth, and early postnatal steroid-resistant nephrotic syndrome (SRNS). Infections, thromboembolism, malnutrition, and psychomotor retardation are common consequences, while terminal renal failure usually occurs at the age of three to eight years [4].

Treatment of CNF is mainly supportive, including daily or every other day albumin infusions, diuretics, replacement of thyroxin and gamma-globulin, a high-protein low-salt diet, and supplements of iron, vitamin D and other vitamins. Prevention of infections and thromboembolic events is also necessary. Angiotensin converting enzyme inhibitors (ACEI) and non-steroidal anti-inflammatory drugs (like indomethacin) are used to decrease proteinuria by reducing intraglomerular pressure. However, some patients need early nephrectomy (even before renal failure develops) due to massive drug-resistant urine protein loss. With known genetic background, immunosuppressive therapy is not advised. However, there are a few reports in literature supporting cyclosporine A (CsA) therapy in hereditary SRNS [5–9]. Here we describe a patient with CNF who responded to CsA by partial remission. Similar experiences from literature are discussed.

Received • Примљено: September 7, 2016
Revised • Ревизија: October 27, 2016
Accepted • Прихваћено: November 29, 2016
Online first: March 14, 2017

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CASE REPORT

The patient is a girl born as the fourth child from the third pregnancy. Birth weight and birth length were 3,650 g and 55 cm, respectively. The placenta was enlarged. Early postnatal development was unremarkable.

The first child from a twin pregnancy and the third child were stillborn due to an unknown cause. Consanguinity was not reported.

Nephrotic syndrome was diagnosed at the age of 2.5 months after DiTePer vaccination. C3 and C4 complements were normal, as well as markers for Epstein–Barr, hepatitis B and C viruses infections. The tests for *Toxoplasma gondii* as well as for *Treponema pallidum* were also negative. Glomerular filtration rate was normal, while severe hypoproteinemia (total protein 37 g/l), hypoalbuminemia (9 g/l), hyperlipidemia (cholesterol 6.3 mmol/l, triglyceride 10.7 mmol/l) and nephrotic-range proteinuria (urine protein/creatinine ratio of 28.2 mg/mg) were found. Kidney biopsy showed immature glomeruli with mild degree of mesangial cell hypercellularity and microcystic dilatation of proximal tubules.

The patient needed daily albumin infusions and diuretics (furosemide and spironolactone). Substitution of vitamin D, thyroxin, and iron, as well as anticoagulants, were regularly administered. In addition, she received gamma globulin replacement and antibiotics during frequent infections. Daily albumin infusions were administered via a central venous catheter (Port-a-Cath), which had to be changed four times due to infections. From the third month of life she has been treated by ACEI and from the seventh month of life she received prednisone without any benefit. After four weeks of not responding to steroids and while waiting for the results of genetic analyses, the patient was started on CsA (150 mg/m^2). She responded with partial remission within three months (urine protein/creatinine ratio of 3.6 mg/mg). Blood level of Neoral was in the 75–150 ng/ml range. Regular albumin infusions were no longer required (Table 1). The results of genetic analyses were finished after nearly a year. The documented homozygous missense mutation in exon 9 of the NPHS1 gene designated as Ex9: c.1048T>C p. (Ser350Pro) was found. CsA continued for the next four years.

At the time this manuscript is prepared the patient is 57.7 months old. Her body height is 100 cm (percentile 8.3, Z score -1.4) and body weight is 16 kg (percentile 25.1, Z score -0.67). She is normotensive, with an average casual blood pressure of 90/60 mmHg. Her serum creatinine is 16 µmol/l and her glomerular filtration rate estimated according to the Schwartz's formula is increased (> 120 ml/min./1.73 m^2) [10]. Total protein and albumin are 56gl/l and 19g/l, respectively. Proteinuria is increased (860 mg/l), with protein/creatinine ratio of 3.2 mg/mg.

DISCUSSION

We reported a patient with CNF due to homozygote missense mutation Ser350Pro. This mutation was described previously [11]. To date, more than 140 different NPHS1 mutations have been identified, comprising nonsense, missense, and frameshift insertion/deletion as, well as splice-site mutations [12, 13]. Clinical presentation and histological findings of our patient were typical for CNF. However, clinical course of the disease was modified significantly by the cyclosporine treatment due to which regular albumin infusions could be discontinued without any negative effects on the renal function. Unfortunately, during the last year, due to unfavorable family situation, she received a lower dosage of cyclosporine that could affect her current proteinuria.

CsA is a calcineurin inhibitor and its antiproteinuric properties are attributed to its immunosuppressive effect related to immunomodulatory action on T cells [14, 15]. In addition, it was demonstrated that the antiproteinuric properties of CsA may also result from a direct stabilization of the podocyte actin cytoskeleton by blocking the calcineurin-mediated dephosphorylation of synaptopodin and upregulating the expression of cofilin-1, which is independent of its effect on synaptopodin [16, 17, 18].

Antiproteinuric effect of CsA in patients with hereditary SRNS varies considerably. The clinical experience

Table 1. Trends of renal function, serum protein and proteinuria over time

Age (months)	sCr (µmol/l)	eGFR (ml/min./1.73 m^2)	serum albumin (g/l)	serum protein (g/l)	Urine protein/creatinine (mg/mg)	Daily iv albumin	Captopril (mg/kgBW/day)	CsA (mg/kgBW/day)
2.5	15	134.7	9	37	28.2	-	-	-
7.3	25	100.8	37	62	32.8	12 g / 24 h	0.4	-
9	28	95	26	55	33	12 g / 24 h	0.4	6.2*
10.5	35–40	76.6	31	60	3.6	12 g / 24 h	0.6	5.8
12	33–83	84.8	31	63	5	-	0.9	4.5
18	26	139.4	23	55	3.6	-	0.7	4.6
20.5	25–40	148	18	53	4.9	-	0.9	7.2
24	21	184.3	18	51	4.9	-	1.2	6.6
30	27	153.3	18	54	4	-	1.2	6.6
42	43–45	103.7		64	3.1	-	0.9	5.4
57.5	16	306.2	19	56	3.2	-	0.9	4.3

sCr – serum creatinine; eGFR – estimated glomerular filtration rate; iv – intravenous; BW – body weight; CsA – cyclosporine A;
*therapy started at the age of eight months

is limited to single-center observations demonstrating a partial response to CsA in selected patients [5–9, 16]. Our patient is an additional case supporting the favorable effect of CsA in CNF. However, because of the small number of reported patients and CsA nephrotoxic side effects, it still remains unclear whether the benefit of CsA-induced partial remission improves overall renal survival. In patients reported by Malina et al. [6], Gellermann et al. [7], Caridi et al. [8], and Hinkes et al. [9], the CsA-induced partial remission significantly improves renal outcome. In contrast, data from a German study strongly support the idea not to expose CNS/SRNS patients with inherited defects related to podocyte function to intensified immunosuppression with CsA [19]. They found a partial remission in only 17% of patients with hereditary CNS (two patients affected by a WT1 mutation) [19]. Preservation of renal function was significantly better in children with nongenetic SRNS after a mean follow-up time of 8.6 years (terminal renal failure in 29% vs. 71%) [19].

The decision to introduce CsA therapy to patients with CNS is even more complicated by the fact that not all recorded NPHS1 mutations had a severe clinical course [12]. The clinical variability is apparently influenced by the sex of a patient, as the majority of mildly affected cases are female [12, 13]. The decision on the introduction of CsA in our patient was encouraged by numerous problems concerning daily intravenous albumin infusions, including technical problems with peripheral veins, or catheter, related infections, and thromboembolic complications. In fact, the CsA therapy was the only alternative to early nephrectomy and consequent renal failure. Also, it should be said that if we had beforehand received the results of genetic analysis, we would probably not have chosen the CsA therapy.

Congenital nephrotic syndrome is steroid-resistant due to an underlying genetic abnormality. Our case demonstrates that CsA can induce partial remission in patients with genetic forms of SRNS without influencing the glomerular filtration rate. However, its long-term effect and safety have yet to be investigated.

ACKNOWLEDGMENT

Writing of this paper was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, grant No. 175079.

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Конгенитални нефротски синдром се може лечити циклоспорином А – приказ случаја и преглед литературе

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САЖЕТАК

Увод Конгенитални нефротски синдром (КНС) испољава се на рођењу или у прва три месеца живота. Фински тип КНС настаје због мутације гена *NPHS1*, који кодира нефрин у подоцитима бубрега. То је врло тешка болест, за коју се не саветује имуносупресивна терапија. Приказујемо болесника са КНС код којег је циклоспорин А (*CsA*) довео до парцијалне ремисије нефротског синдрома.

Приказ болесника Код девојчице од 2,5 месеца испољио се тежак, несиндромски облик стероид-резистентног нефротског синдрома (СРНС). Примењена је агресивна терапија укључујући свакодневне инфузије албумина и диуретике. Добијала је и супституциону терапију тироксина и витамина Д и антикоагулантну терапију, а по потреби, због честих инфекција, гама-глобулине и антибиотике. Лечење инхибиторима ангиотензин конвертујућег ензима није значајно смањило протеинурију. Док смо чекали резултате генетичке анализе, а суочени са бројним проблемима везаним за свакодневне инфузије, честе инфекције и тромбоемболијске компликације, ординирали смо *CsA* као једину алтернативу раној нефректомији и очекиваној бубрежној инсуфицијенцији. Болесник је у току три месеца лечења одговорио парцијалном ремисијом без потребе за инфузијама албумина. После пет година од почетка лечења, њена бубрежна функција још увек није снижена.

Закључак *CsA* може довести до парцијалне ремисије код болесника са генетичким облицима СРНС без негативног ефекта на гломерулску филтрацију. Међутим, дугорочно дејство и сигурност ове терапије треба пажљиво пратити

Кључне речи: мутација гена *NPHS1*; нефрин; стероид-резистентни нефротски синдром; деца

DOI: 10.2298/SARH160824067P
UDC: 616.456.03-055.26

CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Total parenteral nutrition in a twin pregnancy after a suicide attempt with a corrosive substance

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SUMMARY

Introduction Self-poisoning is not frequent during pregnancy. We present a successful treatment of a woman 20 weeks pregnant with twins with self-inflicted poisoning by a caustic substance.

Case outline A 34-year-old pregnant woman was admitted to our institution after self-inflicted poisoning with concentrated acetic acid. Initial clinical evaluation showed severe diffuse erythema of the mouth and oropharynx, a systemic inflammatory response syndrome, and dichorionic diamniotic twin pregnancy in the 20th week of gestation confirmed on abdominal ultrasound. An indirect laryngoscopic examination revealed severe generalized hyperemia of the laryngeal mucosa with corrosive changes in the pharyngeal mucosa, especially of the posterior pharyngeal wall. Due to pain, urgent esophagogastroduodenoscopy could not be performed, and because of the patient's refusal a feeding gastrostomy or jejunostomy could not be created. The patient was given "all-in-one" total parenteral nutrition in addition to other supportive therapy. Gradual introduction of enteral nutrition via a nasoenteric tube placed in the second month of hospitalization failed due to severe vomiting. After almost three months of total parenteral nutrition, enteral nutrition was nevertheless introduced; we then started with oral fluids, increasing gradually to a regular diet, and needed almost half a month to reach the adequate nutritional goal. The delivery was spontaneous at the 36th week of pregnancy and the patient gave birth to two normal healthy girls (46 cm / 2,580 g and 48 cm / 2,960 g, respectively).

Conclusion Total parenteral nutrition can be a safe choice for providing prolonged and adequate nutritional intake even in a twin pregnancy without adverse effects on fetal growth.

Keywords: injury, caustic; pregnancy, twin; nutrition, parenteral, enteral

INTRODUCTION

Suicide attempts have increased significantly and constitute a considerable number of hospital admissions. Acute corrosive poisonings constitute 8–10% of the total number of poisonings in the United States [1, 2]. In the Republic of Serbia the incidence of poisonings with corrosive substances is less than that in the United States, and constituted 2.7% of all cases of poisoning in 2014. However, the number of hospitalized patients due to poisoning with corrosive substances is much higher and constitutes 13% of all admitted patients [3]. These suicide attempts, including self-poisoning, have produced a major sociomedical problem, mostly in young women [4, 5]. Although there are a number of studies on acute intoxication and self-poisoning, studies reporting on pregnant women are limited. Self-poisoning in suicidal attempts is not frequent during pregnancy. The majority of reports of poisoning during pregnancy are case studies and case series of self-poisoning by drug overdose, and few include acute intoxication with corrosive substances [6–13]. In addition to other therapeutic strategies in corrosive injuries, adequate nutritional support must be considered. Also, there are no clear recommendations regarding the type, amount of calories, and duration of clinical nutrition in pregnant women [2]. Although many aspects of the use of total parenteral nutrition (TPN) in pregnancy are controversial, the effects of maternal malnutrition on fetal health are unambiguous [14]. With the inability to apply any kind of nutrition *per via naturalis* including oral food intake or enteral nutrition (EN), TPN must be used to avoid malnutrition, its complications and fetal compromise. We present a successful treatment of a woman 20 weeks pregnant with twins with self-inflicted poisoning by a caustic substance.

CASE REPORT

A 34-year old Caucasian pregnant woman was admitted to our institution eight hours after self-inflicted poisoning with concentrated acetic acid (CH_3COOH). On admission she had difficulty and pain on swallowing, with moderate abdominal pain. Initial clinical evaluation showed severe diffuse erythema of the mouth and oropharynx, blood pressure of 120/70 mmHg, heart rate (HR) of 100 beats per minute (bpm), without clinical signs of respiratory dysfunction and/or aspiration. Laboratory tests showed erythrocyte sedimentation rate of 54 mm/hour, red blood cell count of 3.4 million per μL, white blood cell count of 17,000 per μL, serum

Received • Примљено: August 24, 2016
Revised • Ревизија: January 18, 2017
Accepted • Прихваћено: January 23, 2017
Online first: March 14, 2017

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calcium of 7.3 mg/dL, C-reactive protein of 104 mg/L. Urine testing showed pH of 5.6, a number of fresh red blood cells and 10–15 white blood cells, large amounts of ketones, and hemoglobin. Twenty-four hours after admission the liver transaminase levels started rising and on the fourth day of hospitalization they reached maximum values of 87 IU/L for aspartate transaminase and 245 IU/L for alanine transaminase.

She had no previous psychiatric or medical history, including alcohol or illicit drug abuse, and this was her third pregnancy. Abdominal ultrasound on admission showed a dichorionic, diamniotic twin pregnancy for which the biometric parameters corresponded to the 20th week of gestation: the first fetus had HR of 144 bpm, abdominal circumference (AC) of 138/19.2, and biparietal diameter (BPD) of 50.5, and the second fetus had HR of 149 bpm, AC of 140/19.3, and BPD of 49.4 (Figure 1). An indirect laryngoscopic examination revealed severe generalized hyperemia of the laryngeal mucosa with corrosive changes in the pharyngeal mucosa especially of the posterior pharyngeal wall. Due to pain and erosion in the patient's mouth and pharynx, urgent esophagogastroduodenoscopy could not be performed. Also, a nasogastric tube could not be placed, and feeding gastrostomy or jejunostomy could not be created under local anesthesia due to patient's refusal and the potential risks of bacterial contamination.

After admission we initially started rehydration therapy with crystalloid solutions and low-dose proton pump inhibitors [15–17]. We started prophylactic antibiotic therapy with 1 million units of penicillin G sodium twice a day during the first 10 days of hospitalization. Given the inability to provide adequate nutrition via oral intake or EN, we started TPN. On admission the patient's weight and height were 72 kg and 166 cm, respectively, with a body mass index (BMI) of 26.1 kg/m^2. Before pregnancy, the patient's weight was 54 kg and had a BMI of 19.6 kg/m^2. After the placement of a central venous catheter we started TPN. The patient was given "all-in-one" TPN. The "all-in-one" solution consists of amino acids, carbohydrates, fats, electrolytes, vitamins, elements in traces, and water.

At two weeks after injury oral liquids were reintroduced, but intolerance to oral nutrition remained a reason for TPN continuation. Subclinical deficiencies of magnesium, phosphate, zinc, iron, folate, and vitamin B12 were identified and additional supplies of vitamin D and calcium were also necessary. Our main goal of treatment was to obtain optimal weight gain as for the physiologically fed pregnant woman. Fetal growth was evaluated by ultrasound.

The increase in the maternal metabolic rate was anticipated to be higher than the nutrient intake, but assumed to be compensated by the decrease in physical activity. Fat was given in the „all-in-one" admixture, as essential fatty acids, providing 30% of total calories, and was administered over about 20 hours each day. For determining the continuing nutritional needs and for modifying the quantity of nutrients during progression of the pregnancy, we estimated albumin, transferrin, transthyretin, and daily nitrogen balance values.

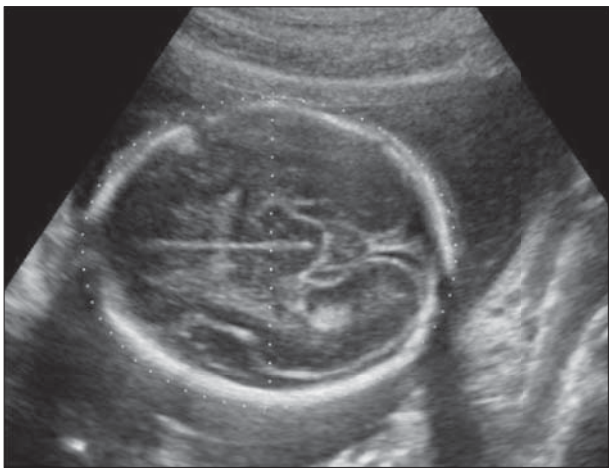

Figure 1. Initial ultrasonography demonstrating 20 weeks of twin pregnancy

Three weeks after caustic injury, an esophagogastroduodenoscopy was performed. Semi-circumferential granulation at the level of the aortic arch and tracheal bifurcation, and circumferential granulation at the level of the lower esophageal sphincter were found, with normal gastric and duodenal mucosa. At the sixth week after injury, a nasoenteric tube was placed. We tried with gradual introduction of EN via nasoenteric tube, but this was unsuccessful due to severe vomiting. From the ninth to 10th week after injury the complete introduction of EN was, however, successful, after which we gradually switched the patient to normal feeding. We started with oral fluids, increasing through nutritious liquids to regular diet, and we needed almost half a month to reach adequate nutritional goals by mouth. During the conversion from TPN to EN and to oral food intake, adequate nutritional intake was provided by TPN and then partial/supplementary parenteral nutrition.

The delivery was spontaneous at the 36th week of pregnancy and the patient gave birth to two normal healthy girls (46 cm / 2,580 g and 48 cm / 2,960 g, respectively).

DISCUSSION

Acute corrosive poisonings have important complications in 18–80% of cases and a mortality rate of 10–38% [1–2]. In our country in 2014, the mortality rate of acute corrosive poisoning was 18.5% [3]. With regard to the gender of patients who died, the majority were women [1–3]. In pregnant women, with the exclusion of unplanned and unwanted pregnancy, probable reasons for suicide attempts could be explained by the many physical, psychological and physiological changes during pregnancy. In a study examining injury and hospitalization during pregnancy, poisoning was reported in 16.4% of cases [18]. The leading mechanism of suicide attempts among pregnant women in California has been ingestion of a drug overdose or corrosive substances [19]. Ingestion of caustic substances leads to systemic and sometimes fatal complications and devastating injuries of the esophagus and stomach. Treatment of these conditions

is quite complicated in itself, and therapy becomes more difficult if the problem occurs during pregnancy.

Ingestion of a caustic substance can produce various injuries to the aerodigestive tract, ranging from very mild to extensive damage, with local, systemic, and long-term complications, often with fatal outcome. In such cases, a multidisciplinary approach and timely treatment could reduce the number of complications and the mortality rate. There are several therapeutic strategies, including adequate rehydration, prevention of aspiration and acute renal injury, antibiotics, surgery if necessary, and treatment of local complications [20–22]. As for other supportive therapies in caustic injuries of the gastrointestinal tract, nutritional support is also important. Decreased maternal protein intake leads to insufficient placental perfusion and fetal compromise. Also, there are adverse effects of maternal ketosis on the fetus [23]. In any case where the patient cannot, should not, or will not eat, TPN must be considered. However, TPN must be applied with great care because of the possibility of TPN-related complications, including pneumothorax, fungemia, venous thrombosis, local infection, elevated liver enzyme levels, fatty infiltration of the placenta, etc. [23, 24].

In the past, TPN during pregnancy has been sporadically employed in the context of a lack of knowledge about maternal–fetal exchange and nutritional requirements during normal pregnancy [25]. The first case of TPN use in gestational hyperlipidemic pancreatitis was described by Weinberg et al. [26]. The patient's symptoms and triglyceride levels were only controlled after initiation of lipid-free TPN; however, the fetus developed intrauterine fetal growth retardation. Adequate nutrition is vital for both mother and child. Delay in giving nutritional support or semi-starving the pregnant women may result in increased fetal mortality and morbidity. Due to our inability to place a nasogastric or nasoenteric tube in our patient we needed to provide parenteral nutritional support. In this case, prolonged parenteral feeding was needed, and a precise evaluation of nutrient needs was difficult. Our main goal of treatment was to obtain weight gain optimal for physiologically fed pregnant women (approximately 4 kg by 20 weeks and 8 kg by 30 weeks of gestation). Because of the higher metabolic rate in our patient there was an increased need of approximately 400 kcal and 40 g of protein over daily basal nutritional requirements. This regimen could logically be increased even further in a twin pregnancy [14, 25]. Our patient was delivered of two healthy neonates with no compromises in fetal growth. We attribute our success to care by experienced clinical nutrition support staff and recommend that TPN in pregnancy should always be managed by such a team.

TPN can be a safe choice for providing prolonged and adequate nutritional intake even in a twin pregnancy without adverse effects on fetal growth. In complicated cases, such as our patient, a precise evaluation of nutrient needs is difficult and adequate monitoring and therapy administration should be managed by multidisciplinary approach and experienced clinical nutrition support staff.

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Тотална парентерална исхрана у близаначкој трудноћи после покушаја самоубиства корозивним средством

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САЖЕТАК

Увод Намерно самотровање није често током трудноће. Приказујемо успешно лечење жене у 20. недељи близаначке трудноће након покушаја самоубиства каустичним средством.

Приказ болесника Трудница у доби од 34 године примљена је након намерне ингестије концентроване сирћетне киселине. Иницијална клиничка процена указала је на изражен дифузни еритем уста и орофаринкса, синдром системског инфламаторног одговора и дихорионско диамнионску близаначку трудноћу у 20. недељи гестације утврђену ултразвучним прегледом. Ларингоскопијом је утврђена тешка генерализована хиперемија ларингеалне слузнице са корозивним променама на фарингеалној слузници, нарочито у пределу задњег зида фаринкса. Хитна езофагогастродуоденоскопија није урађена, а ни нутритивна гастростомија и јејуностомија због одбијања труднице. Ординирана је тотална парентерална исхрана (ТПИ) „све у једном“ и остала супортивна терапија. Постепено увођење ентералне исхране (ЕИ) преко назоентералне сонде пласиране у другом месецу хоспитализације било је неуспешно због израженог повраћања. Након три месеца примене ТПИ, уведена је ЕИ, после чега је отпочето давање течне хране, са постепеним увођењем регуларне исхране на уста, што је трајало готово пола месеца како би се достигле адекватне нутритивне потребе. Порођај је био спонтан у 36. недељи трудноће и рођене су две нормалне здраве девојчице (46 *cm* / 2.580 *g* и 48 *cm* / 2.960 *g*).

Закључак ТПИ може бити сигуран избор у продуженом обезбеђивању адекватне количине нутритивног уноса и у близаначкој трудноћи без нежељених ефеката на фетални раст.

Кључне речи: повреда, каустична; трудноћа, близаначка; исхрана, парентерална, ентерална

DOI: https://doi.org/10.2298/SARH161228107N
UDC: 616.351-006-085

CURRENT TOPIC / АКТУЕЛНА ТЕМА

Road to organ preservation in locally advanced rectal cancer

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SUMMARY

Introduction In the past 20 years there has been significant change in the treatment of rectal cancer, especially in terms of multimodal approach. Surgery is, at least for now, the mainstay treatment for resectable rectal cancer. Preoperative chemoradiotherapy is, regardless of its modality, short or long course, different chemotherapeutic regiments, widely recommended for locally advanced rectal cancer. After neoadjuvant treatment, 15–27% of patients experience pathological complete response (pCR). These patients could benefit from non-operative management, thus avoiding potential surgical complications and possible reduction in the quality of life. Unfortunately, one cannot precisely define, while omitting surgery, which patients have pCR. For this reason Habr-Gama, a pioneer in the "watch-and-wait" strategy, developed a new endpoint for non-operative management – clinical complete response. To measure the response, in the absence of pathological examination, same diagnostic tools are used as in initial staging, but none is reliable enough to be used alone.

This article is focusing on critical points in the reassessment of response to preoperative chemoradiotherapy for advanced rectal cancer, which is mandatory for appropriate selection of patients who might benefit from non-operative management.

Keywords: rectal cancer; organ preservation; non-operative management; chemoradiation therapy; total neoadjuvant therapy, clinical complete response; pathologic complete response

INTRODUCTION

Surgery is, at least for now, the mainstay treatment for resectable rectal cancer. Anatomic description of total mesorectal excision emphasizing mesorectum, mesorectal fascia, and circumferential resection margin introduced by Heald et al. [1] in 1982 and the implementation of this technique, managed to reduce the incidence of local recurrence. In cases of locally advanced rectal cancer radiotherapy combined with surgery results improved in terms of local recurrence, and according to a Swedish trial even overall survival improved [2, 3]. Fluorouracil based chemotherapy was added for radiosensitising. According to a meta-analysis which included five studies, preoperative administration of combined chemo and radiotherapy offers better results than preoperative radiotherapy alone at five years in terms of local recurrence ($p < 0.001$), but without statistically significant difference in disease-free survival ($p = 0.27$) or overall survival ($p = 0.58$) [4]. A German rectal cancer study demonstrated superiority of preoperative administration of radiotherapy with concurrent chemotherapy, in comparison to the same regiment applied in the postoperative setting in terms of five-year local recurrence ($p = 0.006$), but also without statistical difference in overall survival (OS), disease free survival (DFS) and distant recurrence [5]. Fluorouracil-based chemotherapy is most widely used in a neoadjuvant setting, although in search of an ideal radiosensitizing agent, other drugs, such as oxaliplatin, capecitabine, and irinotecan, are being tested [6].

According to published data there seem to be no benefits from postoperative administration of fluorouracil-based chemotherapy in patients who already received preoperative chemoradiotherapy, since it doesn't offer better results in terms of local recurrence, OS, and DFS [7, 8]. The long-term results from of the EORTC 22921 study, after a median follow-up of 10.4 years, confirmed these results [9].

In order to clear the dilemma regarding short-course and long-course radiotherapy, a systematic review of 16 trials (12 in meta-analysis) was conducted in 2014. The authors concluded that there is no difference in local recurrence, DFS, and OS between patients treated with short-course preoperative radiotherapy with immediate surgery and long-course preoperative chemoradiotherapy, suggesting that short-course radiotherapy could be more convenient in centers with longer waiting lists or lack of medical resources [10].

Given these oncological results, regardless of its modality, short or long course, or different chemotherapeutic regiments, preoperative chemoradiotherapy is widely recommended for locally advanced rectal cancer.

Received • Примљено: December 28, 2016
Accepted • Прихваћено: April 11, 2017
Online first: April 21, 2017

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RESPONSE TO PREOPERATIVE CHEMORADIOTHERAPY

After preoperative chemoradiotherapy, 15–27% of patients have pathological complete response (pCR). According to Quah et al. [11], pCR is absence of any viable tumor cells in the resected specimen, irrespective of the proportions of necrosis and fibrosis. It can also be measured as tumor response grade (TRG) from 0 to 4 [12]. Some studies use the Mandard grading, which is adopted from the measurement of response in oesophageal cancer (grades from I to V). According to long-term results from CAO/ARO/AIO-94 trial, 10-year DFS for patients with TRG 4 is 89.5%, while for those with TRG 0 it is 1–63%. According to multivariable analysis, residual lymph node metastasis (ypN+) and TRG are independent prognostic factors for cumulative incidence of distant metastasis and DFS ($p = 0.039$) [13]. Similar results were published in 2008 on 119 patients treated with preoperative chemoradiotherapy for locally advanced rectal cancer, showing pCR of 14.2% [14]. In this study, response grades I or II according to Mandard are good indicators of DFS and are better prognostic factors than down-staging. The data from pooled analysis on 3,105 patients corroborated these results, showing five-year DFS of 83.3% for patients with pCR, and 65.6% for those without pCR ($p < 0.0001$), which could be the result of biological characteristics of the tumor [15].

Patients with pCR might be overtreated with surgery and there is a trend for strict surveillance and organ preserving in these cases. Unfortunately, we cannot precisely define while omitting surgery which patients have pCR. For this reason, Habr-Gama et al. [16] developed a new endpoint for non-operative management – clinical complete response (cCR), which is absence of clinically detectable residual primary tumor.

In a study from UK on 129 patients from two centers, only one third of patients who were deemed with cCR actually had pCR according to the Mandard classification [17]. The authors explain their reported rate of pCR (10.1%) with a different chemoradiotherapy protocol and with the interval to surgery, which was within four to eight weeks, since it is recognized that waiting beyond this point could result in better response [17]. Escalating radiation doses may also have influence on tumor response but at the same tame could compromise functional outcome [18]. The role of other radiotherapy techniques in improving response is beyond the scope of this paper.

In 2016, two meta-analyses were published on the subject of interval to surgery, with pCR as the primary endpoint, while DFS, OS, and sphincter preservation were secondary endpoints. A meta-analysis from Italian authors included 13 prospective and retrospective studies with 3,587 patients [19]. According to their results, pCR improved by 5.8% when the interval to surgery was longer than six to eight weeks, without compromising OS and DFS and with similar complication rates and sphincter preservation. A systematic review and meta-analysis by Wang et al. [20] included 15 retrospective studies with 4,431 patients and pCR ranging from 8.3% to 28%. The highest pCR rates were recorded in patients operated on beyond eight weeks after the end of chemoradiotherapy, which was associated with an approximately 49% higher chance for pCR compared to patients who were operated on earlier. Prolonging the interval beyond 10 or 12 weeks did not offer further advantages and also didn't affect survival or rate of sphincter spearing procedures [20].

PREDICTORS OF RESPONSE

A number of retrospective studies were undertaken in an attempt to identify predictive factors of response to neoadjuvant treatment using simple blood tests (hemoglobin, Ne/Ly ratio, albumin, and fibrinogen), biomarkers (Ki67 and thymidylate synthase and EGFR expression, wild-type p53 status, microRNA, etc), morphological characteristics of the tumor, and the distance from the anal verge or certain imaging features [21–28]. Few of them are reproducible. Results from several studies showed that the N stage is a predictor of response to preoperative chemoradiotherapy [23, 29]. According to Russo et al. [30], the absence of mutation of commonly mutated cancer genes may be associated with a higher likelihood of having a pCR. In the same study, the level of CEA ≤ 2.5 and smaller tumor size were predictive factors of pCR. Other studies have also found decreasing tumor size to predict response, thus suggesting it should be considered as a valid parameter for selecting patients for organ preserving [29]. The level of CEA either at diagnosis or post-chemoradiotherapy is also an independent risk factor for response according to several retrospective studies [28, 29, 31]. A recently published study by Probst et al. [32], which included data on 18,113 patients retrieved from the National Cancer Database, showed that high CEA at diagnosis was independently associated with decreased pCR response ($p < 0.001$), pathological tumor regression ($p < 0.001$), tumor downstaging ($p < 0.001$), and OS ($p < 0.001$). According to these results, patients with increased pretreatment levels of CEA are not good candidates for organ preservation.

CRITICAL POINTS IN REASSESSMENT

In an ideal scenario, one could be able to identify patients with complete response in the restaging process and select patients for non-operative management, thus avoiding operation and possible early or late morbidity, reduction in the quality of life, especially in cases where permanent colostomy is needed. For reassessment, in the absence of pathological examination, the same diagnostic tools are used which were available for initial staging [digito-rectal examination (DRE), proctoscopy, and imaging techniques]. Concordance between DRE and pathologically based assessment of response to preoperative chemotherapy was investigated in a prospective study by Guillem et al. [33] in 94 patients with locally advanced rectal cancer. After a median interval of 48 days from the completion of therapy, the patients were referred to surgery and under anesthetics the same surgeon who performed the initial

assessment performed comprehensive DRE. DRE underestimated the response in 73 patients (78%), overestimated it in none, and was able to identify only 21% of patients with pCR. The overall concordance of DRE and pathologic response was only 22%. The specificity of clinical examination in determining complete or near-complete pathologic response (≥ 90% tumor regression) was 56%, the sensitivity was 24%, and positive and negative predictive value was 19% and 61%, respectively, while the accuracy was 49%.

Proctoscopy further allows visual confirmation of digital findings. Habr-Gama et al. [34] provided a comprehensive overview of clinical and endoscopic features in cCR and proposed further standardizations. According to this overview, any residual finding needs surgical attention, from excision to more radical surgery, while biopsies are not recommended. Patients with cCR should have no more than whitening of the mucosa, telangiectasia with mucosal integrity to be considered for the organ preserving approach [34]. However, data from a retrospective study conducted by Smith et al. [35] showed that only 16 out of 61 patients with pCR had mucosal irregularity and by that fulfilled the criteria for cCR. On the other hand, six out of 22 (27%) patients with mucosal cCR still had the residual disease. Han et al. [36] also tried to determine the correlation between endoscopic findings and ypT in a retrospective study which included 481 patients. Pathological good response was defined as ypT ≤ 1. Patients were randomized either into the testing or the validation group. The validation was done using endoscopic findings determined in the testing group. Endoscopic features that correlated with good pathological response were scaring, telangiectasia, and erythema, while nodule, ulcer, stricture, and remnant tumor were signs of minimal or no response. The kappa statistic for interobserver model was 0.965. This classification system showed high specificity and negative predictive value but low sensitivity and positive predictive value, implying that it can strongly predict patients with minimal or no response but is less able to identify good response. They further suggest that these criteria could be helpful in selecting candidates for local excision (LE) [36].

Whether or not local excision is necessary is still debatable. Issa et al. [37] reviewed results from 31 patients with cCR who underwent LE (transanal excision or transanal endoscopic microsurgery) after neoadjuvant chemoradiotherapy for locally advanced rectal cancer. Twenty-three patients had ypT0 while in eight patients the residual disease was found. After median follow up of 87 months, three patients died from other causes. No distant or local recurrences were observed in remaining patients [37]. Accurate selection of patients for LE is still lacking, while salvage radical surgery can be challenging [38]. A recent systematic review and meta-analysis compared the outcome of patients after preoperative chemoradiotherapy followed by LE, with patients who had radical surgery after neoadjuvant treatment [39]. Local recurrence rate was higher with LE, although it didn't reach statistical significance (p = 0.40). There was no difference in 10-year OS (p = 0.93). The same results were obtained for the subgroup with T3 / any N stage tumors. After LE, the status of the mesorectal lymph nodes remains unknown. The reported median rate of lymph node metastases in patients with pCR is 7%; thus, mucosal response should not be the single factor for patient selection. Patients with understaged nodal involvement and LE have poorer outcome, since lymph node status is the most important prognostic factor in rectal cancer. The biggest challenge is to adequately evaluate lymph node status after preoperative chemoradiotherapy and this is the basis for criticism in organ preserving [40].

Reassessment is further performed using imaging techniques like computed tomography (CT), endorectal ultrasound or magnetic resonance imaging (MRI). Conventional MRI is less accurate for reassessment than initial staging, mostly due to the difficulty in distinguishing fibrosis, oedema, and normal mucosa from small foci of a residual tumor [41]. According to a meta-analysis, conventional ultrasound and MRI are unreliable for both T and N stage. In T2-weighted imaging, fibrous tissue as a result of chemoradiotherapy may be indistinguishable from the tumor [42]. Diffusion-weighted imaging MRI (DWI-MRI) is helpful in distinguishing residual viable tumor from treatment-related changes and can depict microstructural and metabolic treatment-induced changes of the tumor before morphological changes become apparent. It allows performing quantitative measures such as apparent diffusion coefficient (ADC), which may be useful as an imaging biomarker of tumor characteristics [43]. In order to investigate the added value of qualitative DWI-MRI evaluation in assessment and to evaluate the diagnostic performance of ADC measurements, Foti et al. [44] conducted a single-institution study including 31 patients with locally advanced rectal cancer. The pCR rate was 16.1%. According to their results, diagnostic performance of added DWI-MRI to conventional MRI was better than MRI alone. Sensitivity improved from 20% to 80%, negative predictive value from 87.5% to 96.6%, and accuracy from 87.9% to 99.6%. In three cases the interpretation of additional DWI-MRI allowed corrections of diagnostic errors made on the basis of conventional MRI interpretation alone, differentiating viable tumor from fibrosis. Additionally, according to their results, pretreatment examination ADC value has a potential to predict treatment response, suggesting that the change in ADC values has the potential to provide a surrogate biomarker of treatment response in rectal cancer [44]. Guillem et al. [45] in a prospective study compared the ability of fluorodeoxyglucose positron emission tomography (FDG-PET) and CT in detecting pCR, whose rate was 21%. These procedures failed to adequately distinguish a pCR from an incomplete response; also none of the PET parameters like mean or standard uptake value, total lesion glycolysis, are accurate for distinguishing pCR from incomplete response [45]. In a paper by Joye et al. [46], 14 relevant studies on the role of DWI and FDG-PET/CT in the assessment of pCR after chemoradiotherapy were systematically reviewed. Pooled analysis showed that qualitative DWI assessment had a higher accuracy in predicting pCR than quantitative analysis (87% vs. 74–78%), but sensitivity of ADC measurements are higher than qualitative DWI assessment (78–80% vs. 53%).

Quantitative and qualitative FDG-PET/CT has a similar predicting response. The ability of functional imaging to predict pCR is affected by the interval between the end of chemoradiotherapy, reassessment, and surgery. Generally, a low pretreatment ADC, an increase in ADC, and a decrease in standardized uptake value are associated with better response to radiochemotherapy. Pooled analysis shows that qualitative DWI assessment 5–10 weeks after the end of radiochemotherapy outperforms ADC-based DWI parameters. They conclude that DWI and FDG PET/CT are not accurate enough to safely select patients for organ preservation [46].

ROAD TO ORGAN PRESERVATION

Several studies published their results with watch-and-wait policy, including the pioneering work of Habr-Gama, with promising results in terms of oncological safety, although most are retrospective in nature [47–51]. Conclusions are similar: larger number of patients included in prospective analysis is required, longer follow-up is needed, and selection criteria must be strict, as well as the protocol of surveillance. In the largest study published so far, 229 patients with surgical resection after preoperative chemoradiotherapy and 129 patients with cCR who were managed with watch-and-wait were matched for the T stage, age, and performance status (109 patients in each group) [52]. More than 60% of patients in the watch-and-wait group avoided major surgery without compromising oncological safety, compared to the group with surgical resection. Patients managed by the watch-and-wait strategy had a significantly better three-year colostomy-free survival rate than those who had surgical resection. Reassessment after preoperative chemoradiotherapy and selection of patients who might benefit from the watch-and-wait strategy still remains a critical issue.

Although advantages of the watch-and-wait strategy are reduced stoma requirements, improved functional results, and avoidance of major surgery, this approach has its weakness. Disadvantages over surgery are the following: difficulties in determining clinical stage 0, follow-up is imperative, as is surgeon–patient confidence [53]. It's rather difficult to conduct randomized trial in a situation where informed patients would have their own preferences.

In lack of randomized control trials and in order to provide solid evidence on organ preservation in rectal cancer, in 2014, a group of experts following their meeting in Lisbon created the International Watch & Wait Database (IWWD), which should provide more information on individualized risk with this approach. This is especially important for motivated patients who are willing to trade unknown oncological risk for a good quality of life. The decision making process is less complicated for high-risk elderly patients than for the young and fit [54]. The results are awaited.

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Пут ка презервацији органа код узнапредовалог карцинома ректума

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САЖЕТАК

У последњих двадесетак година дошло је до значајних промена у лечењу карцинома ректума. Хирургија представља методу избора у лечењу ресектабилног карцинома ректума. Преоперативна хемиорадиотерапија је широко прихваћена у лечењу локално узнапредовалих тумора ректума. Након неоадјувантне терапије код 15–27% болесника долази до комплетног патолошког одговора. Ови болесници могу имати користи од неоперативног лечења, избегавајући потенцијалне хируршке компликације и могуће смањење квалитета живота. Нажалост, не може се прецизно, без операције, дефинисати комплетан патолошки одговор. Из овог разлога је Хабр-Гама развила нови циљ неоперативног лечења – комплетан клинички одговор. За процену одговора, у одсуству патохистолошког налаза, користе се исти дијагностички поступци као и при иницијалном стадирању, али ниједан није довољно поуздан да би се користио самостално. Овај рад се фокусира на критичне моменте у процени одговора на преоперативну хемиорадиотерапију код узнапредовалих карцинома ректума, која је неопходна у правилном одабиру болесника који могу имати користи од неоперативног лечења.

Кључне речи: карцином ректума; презервација органа; нехируршко лечење; хемиорадијација; тотално неоадјувантно лечење, клинички комплетни одговор; патолошки комплетни одговор

DOI: 10.2298/SARH170222101K
UDC: 61(091)(497.11)"18"; 61:929 Сибер Ј.; 615:929 Прикелмајер К.

ИСТОРИЈА МЕДИЦИНЕ / HISTORY OF MEDICINE

Ваљевски лекар Јован Сибер и апотекар Клаудије Прикелмајер – историјска скица о улози досељеника из Славоније на развој здравства у Србији

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САЖЕТАК
У кратком раздобљу током друге половине шездесетих година 19. века у Ваљеву су основане три нове значајне институције: болница (1867), гимназија (1869) и апотека (1870). У почетном раду тих институција велику улогу су имали досељеници из Славоније: лекар Јован Сибер, апотекар Клаудије Прикелмајер и професор Ђуро Козарац.
Овај рад има за циљ да разјасни међусобне везе ових личности, које су утицале на њихов долазак са територије Хабзбуршке монархије у Србију и Ваљево, као и да разреши забуне до којих је дошло на основу непоузданих породичних сећања и хроничарских записа. Истовремено, у раду је додатна пажња посвећена и пореклу лекарске породице Сибер (*Sieber*), чији је члан био Јован Сибер, и накнадном доласку у ваљевски крај других чланова исте породице, међу којима је био и његовог синовац др Стеван Сибер, отац каснијег санитетског генерала војске Краљевине Југославије др Ђорђа Сибера.
Кључне речи: историја медицине; XIX век; медицина; хирургија; Србија

УВОД

Током историјског процеса стварања нововековне државе у 19. веку у Србију су се досељавали бројни странци, који су својим знањем и делом дали велики допринос њеном даљем развоју. Они су долазили подстакнути пансловенском и југословенском идејом, али и у потрази за послом и развојем каријере, чиме су представљали својеврсну економску емиграцију, али не као необразовани појединци, спремни на било какве послове, већ као стручњаци који су желели да каријеру почну или наставе у окружењу у коме је конкуренција знатно мања него у њиховој матичној држави. У потрази за бољим послом, могућношћу напредовања и веће зараде, образовани кадрови су у Србију долазили пре свега из Хабзбуршке монархије. Међу њима су били припадници различитих нација који су живели на територијама насељеним словенским становништвом и у новој средини нису наилазили на велику језичку баријеру. Многи од њих су се врло брзо уклопили у локалну средину, тако да су се следеће генерације лако асимиловале. У почетку су поред школованих стручњака долазили и неадекватно образовани, али са знањем које је у Србији поседовао недовољан број појединаца. Тако, примера ради, 1829. године из Баната у Србију долази извесни Јован Илић. Он је по занимању био столар, али образовање које је имао омогућило му је да нађе посао учитеља почетних разреда у тек основаној школи у Бранковини [1]. Како је писао један од његових првих ученика Константин Ненадовић: „Доста знања за почетног учитеља притеживао је“ [2]. Поред других професија, налазећи послове који су превазилазили њихово образовање и које не би добили у државама са конкуренцијом адекватно образованих, налазили су се и приучени лекари, емпиријци, попут Григорија Рибакова и Антона Делинија, који су током тридесетих година 19. века службовали и у Ваљеву као општински физикуси [3]. Описујући своје образовање у писму које је 28. августа 1833. године (по јулијанском календару, по коме су презентовани сви датуми сем оних за које је наглашено да су по грегоријанском календару) упутио кнезу Милошу Антон Делини пише: „Двадесет сам година у Србији провео и себи великим трудом и лекарним покраи мале теорије читањем медицески на Талијанском и Греческом језику списаних књига, а нарочито дуговременим искуством“ [4].

Временом, са развојем и модернизацијом Србије, стање се мењало и неадекватно образовани више нису могли да проналазе пословне ангажмане. Тако, када је први ваљевски апотекар Клаудије Прикелмајер крајем 1870. године упутио молбу министру просвете и црквених дела да буде постављен за професора нотног певања у

Примљено • Received: February 22, 2017
Прихваћено • Accepted: March 22, 2017
Online first: April 11, 2017

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тек основаној Ваљевској гимназији, одбијен је пошто није поседовао формалне квалификације за то место, упркос доброј практичној музичкој вештини [4]. Када се млада држава овако односила према подучавању нотног певања, очекивано је да се на исти начин, па и још строже, односи и према лекарској делатности. Тако већ крајем шездесетих година 19. века за положај окружног физикуса не само да нису могли да конкуришу аутодидакте већ ни школовани лекари који су у школама Хабзбуршке монархије достигли најнижи степен – патрон хирургије. Међутим, због недостатка лекара са вишом степеном образовања спремних да се ангажују у мањим местима, увек је било изузетака. Међу такве изузетке спада и патрон хирургије Јован Сибер, који је од 1869. до 1874. био ваљевски окружни физикус и управник тек основане ваљевске болнице. Занимање за активности Јована Сибера отвара нове истраживачке хоризонте усмерене ка осталим члановима његове породице и њиховим везама са другим значајним досељеницима из Славоније током друге половине 19. века, укључујући и поменутог апотекара Клаудија Прикелмајера.

По сећањима др Ђуре Прикелмајера, најмлађег Клаудијевог сина, његов отац је радећи у Ђакову, у туђој апотеци, свирао оргуље у катедрали код славонског бискупа Јосипа Јураја Штросмајера, и једном приликом, за време ручка, бискуп му рече: „Знаш синко, моја је жеља да ти сада службујеш негде у Кнежевини Србији“ [5]. Са друге стране, пишући о оснивању Ваљевске гимназије и улози коју је у томе имао Ђуро Козарац, католички свештеник и професор филозофије у Ђакову, Љубомир Павловић је записао: „У Ваљево није случајно дошао. Заволео је још у Пожези Ему, кћи Павла Сибера, тамошњег апотекара. С њом је побегао у Србију и наишао на своме путу на свога друга из школе Клаудија Прикелмајера, апотекара из Ваљева. И њега и вереницу Прикелмајер је на својим колима довео у Ваљево. Вереницу преда њеном стрицу др Стевану Сиберу, лекару ваљевском, а Ђуру одведе у свој дом“ [6].

ПОРЕКЛО ПОРОДИЦЕ СИБЕР

Наведено сведочење отвара питање везано за име ваљевског лекара Сибера: Јован или Стеван? Трагајући за његовим пореклом, налазимо да је у Славонију у 19. веку радио извесни лекар Јосип Сибер (*Sieber*). По породичним сећањима, његова породица је однекуд из Немачке стигла у Славонију током друге половине 18. века, а он је рођен у Валпову 1782, где је и умро 1862. године. Током тридесетих година 19. века основао је Валповачке Топлице [7]. У Валпову су рођена његова три сина, сви лекари: Јосип (1813–1870), који је радио у Ђакову, Иван – Муки (1819–1865), „који се преселио у Србију – у Ваљево“ и Едуард (1832–1905), који је остао у Валпову и наставио са развојем и модернизацијом Валповачких Топлица [8].

Сада би требало да ове три различите али повезане приче настале на основу породичних сећања обједи-

нимо у једну целину, али и да разрешимо више недоумица. Прво можемо да закључимо да је Иван Муки Сибер онај поменути ваљевски лекар Јован. Што се тиче времена досељавања Сибера у Славонију и места рођења валповачког лекара Јосипа и његова два старија сина, расположива грађа указује на чињенице донекле другачије од оних забележених на основу породичних сећања. У ђаковачким матичним књигама умрлих, до којих смо дошли посредством адвоката Жељка Лекшића, забележено је да је др Јосип Сибер, који је умро 28. јула 1870. године (по грегоријанском календару), рођен у „Kisch in Bohemia“ [9], а исти податак о месту рођења – „Bohemus“ забележен је и на његовој лекарској дипломи [10]. Што се тиче места рођења његовог брата Ивана, односно у Србији Јована, доступне су његове кондуит листе из 1856. и 1874. године, на које нас је упутио др Александар Недок и у којима пише да је и он рођен у Кишу у Чешкој, 1819. године [11, 12]. Што се тиче најмлађег брата Едуарда, по подацима забележеним у валповачкој матичној књизи крштених, рођен је 15. марта (по грегоријанском календару) 1832. године, у Валпову [7].

На основу наведених информација можемо да закључимо да је у породичним сећањима наследника породице Сибер дошло до заборава и да др Јосип старији није рођен у Валпову, већ се доселио са територије Чешке, где су му претходно рођена два старија сина, Јосип млађи и Иван. У Славонију је стигао током двадесетих година 19. века, будући да је забележено да је Иван гимназију учио у Осијеку [11, 12], а у Валпову му је рођен најмлађи син Едуард (1832). Док је Едуард, због касног рођења могао да дочека пензионерске дане свога оца (умро 1862), и да настави његову праксу у малом Валпову, којем није било потребно више лекара, за старију браћу није постојала та могућност. Зато је најстарији син Јосип радио у Ђакову, а средњи – Иван своју је каријеру везао за Србију, делом конкретно за Ваљево, и то управо у времену када је основана Ваљевска болница.

ОСНИВАЊЕ ВАЉЕВСКЕ БОЛНИЦЕ И АПОТЕКЕ

Ваљевска болница је формално основана 31. јануара 1867. године, када је по добијању дозволе Министарства унутрашњих дела начелство округа ваљевског потписало уговор са трговцем Алексом Андоновићем о закупу његове спратне куће, у „новој чаршији“ (улица Кнеза Милоша) за потребе болнице [3, 4]. Управник болнице је по аутоматизму био дотадашњи окружни физикус Франц Тесар, али исте године он је напустио Ваљево, а заменио га је магистар хирургије и ветерине Франц Бихеле [3, 4, 13], који се такође у Ваљеву није дуго задржао. Већ следеће, 1868. године, 27. августа, у Српским новинама објављен је оглас Министарства унутрашњих послова за пријем већег броја лекара: „У Књажевству Србији има више празних места за среске и варошке лекаре и за лекарске помоћнике при шпиталима. Среска и варошка места попуњавају се док-

торима медицине или магистрима хирургије, а она за лекарске помоћнике при шпиталима патронима хирургије. Који жели једно од ти места заузети, нека се најдаље до конца месеца септембра ове године обрати министру унутрашњих дела, и поднесе диплому, заједно са осталим сведочанствима и у писму свом изложи потпуно националне, као и то је ли где служио и где сад практикује" [14]. Да је један од градова за које се тражи лекар било и Ваљево, види се из огласа који је Општина ваљевска објавила пола месеца касније, 10. септембра: „Општина ова имаде одобрење од власти да може у својој вароши отворити и јавну апотеку под условима који су изложени у &. 6. Закона за апотеке и апотекаре. Зато према овоме позива свакога који ово жели отворити, и за себе радити, да се овоме Суду с нужним документима обрати, од данас, па за један месец дана те да му општина ово своје право под условима реченог закона даде; покрај тога потребује јој и лекар, коме ће плаћати 300 талира годишње плате. Дакле, који би ово званије желео получити код ове општине такође нек се Суду овоме до означенога рока јави. Но прије свега позива нарочито оне лекаре који би желели од овог двог и једно и друго примити, а имају диплому магистра фармације, а у противном случају да ово на себе узети могу, па ма сами себи набавили магистра фармације као администратора отворене апотеке" [15].

По свему судећи, Бихеле је већ половином 1868. године напустио Ваљево или исказао намеру да оде, и општинске власти су тражиле новог лекара, али и апотекара. Оснивање болнице створило је реалну потребу да поред уобичајених лекарских приручних апотека у Ваљеву постоји и засебна апотекарска институција. Зато се општина обратила државним властима и средином 1868. године добила адекватне дозволе [4, 16]. Тако се већ од јесени 1868. године у Ваљеву очекивао долазак новог лекара, али и првог варошког апотекара. Међутим, за попуњавање оба радна места постојали су објективни проблеми. У Србији је у том тренутку било врло мало магистара фармације, као и лекара са звањем магистра хирургије или доктора медицине који су били спремни да оду у провинцију и да уз посао општинског физикуса волонтерски врше и функцију управника болнице, што је можда био и разлог брзог одласка и кратког боравка Тесара и Бихелеа. Зато се, мимо прописа, толерисало да се на место окружног физикуса постави лекар са нижим звањем, и из Горњег Милановца у Ваљево долази патрон хирургије Јован Сибер.

ДОЛАЗАК ЈОВАНА СИБЕРА

Одазивајући се на расписани оглас, Сибер је у контакт са Ваљевском општином ступио крајем 1868. или на самом почетку 1869. године. Преузимајући посао окружног физикуса, предложио је и да се проблем недостатка апотеке реши уз помоћ његове приручне апотеке, која би се третирала као општинска. То се види из притужбе коју је 4. фебруара 1869. године Министарству унутрашњих послова упутио апотекар Мијаило Филиповић, жалећи се да ваљевска општина није прихватила предлог да он у Ваљеву отвори апотеку, већ се договорила са Сибером о коришћењу његове приручне апотеке. Министарство није позитивно одговорило на ову притужбу, зато што, по тадашњим важећим прописима, једно лице није могло да има две апотеке, а Филиповић је већ водио апотеку у Шапцу [4].

За патрона хирургије Јована Сибера у његовим кондуит листама, поред поменутог податка да је рођен 1819. године у Кишу, забележено је да је гимназију учио у Осијеку, а да је у Прагу завршио „медико-хирургическу науку, диплому има хирургије", да је пре доласка у Србију „у цесарско-краљевској војски био лекар војени од 1845. до 1852", да је у Србију дошао 16. децембра 1852. са 33 године, да је прво радио у Ћуприји, па од 1962. у Краљеву, затим у Горњем Милановцу и Ваљеву. Био је врло пријатна, интелигентна и савесна личност, добре нарави, учтив и без порока, која зна добро српски и у читању и у писању, а поред њега немачки, латински и италијански, али и да је „доста слаб и нејак, као стар човек". Ова опаска је наглашена у листи из 1874. године, када је имао 55 година [12]. Те године је и пензионисан, а умро је у Ваљеву следеће, 1875. године, 7. маја [3]. У поменутој кондуит листи записано је да је Јован био ожењен извесном Славком Радуловић, са којом је имао двоје деце – мушко и женско. О њима данас нема познатих сачуваних информација, а у забележеним породичним сећањима постоји опаска да Јован није имао наследника, а да је из породице Радуловић потекао и проф. др Бранко Радуловић, ортопед, оснивач Института Бањица [10].

По свему судећи, Јован Сибер је у Србију дошао зато што као војни лекар са најнижом звањем патрона хирургије није имао могућност за напредовање у санитетској хијерархији војске Хабзбуршке монархије, док је у Србији могао да добије државно запослење, уз могућност обављања и приватне праксе. Међутим, са развојем Србије, са приливом нових кадрова и модернизацијом законодавства, формално није могао да буде окружни физикус и управник болнице, али фактички, у недостатку других кандидата, то му је омогућено, чему су, могуће је, допринеле и позитивне оцене његове личности и претходног рада. Међутим, одредбе Закона за апотеке и апотекарство из 1865, које су онемогућиле шабачком апотекару Филиповићу да у Ваљеву отвори другу апотеку, онемогућиле су и поменути договор да се Сиберова приручна лекарска апотека, а без ангажмана магистра фармације, уступи вароши као јавна. Зато су ваљевске власти актуелизовале потрагу за апотекарем и убрзо на град у Колубари стиже Клаудије Прикелмајер.

ДОЛАЗАК КЛАУДИЈА ПРИКЕЛМАЈЕРА

Прикелмајер (1837–1913) био је родом из Славонске Пожеге (Слика 1). Фармацију је студирао у Бечу, где је дипломирао 1863. године, када се враћа у Пожегу,

Слика 1. Клаудије Прикелмајер у својој апотеци у Ваљеву, око 1911. године [4]
Figure 1. Klaudije Prikelmajer in his pharmacy in Valjevo, circa 1911 [4]

а краће време борави у Ђакову, о чему сведоче фотографије које је ту сам начинио 1864, а на којима су портрети виђених Ђаковчана, међу којима је и бискуп Штросмајер, али и млади чланови и чланице локалног певачког друштва. Потом се враћа у Беч на додатну праксу, а 1867. поново долази у Ђаково и ради у апотеци тамошњег апотекара [4, 17, 18]. Како смо већ нагласили, а на основу породичних сећања, Прикелмајер је у Србију дошао по директном наговору бискупа Штросмајера [5]. Било му је понуђено да апотеку отвори у Неготину или Ваљеву, а Ваљево је одабрао „као место најближе Београду, али и Славонији" [4, 16].

Прича о директном утицају бискупа Штросмајера на Клаудијев долазак у Србију се не сме одбацити, мада је могла да настане као резултат накнадних романтичарских пројугословенских ставова, али се морају размотрити и други утицаји, који нису у колизији са наведеним. Могло би да се претпостави да је на Прикелмајеров долазак из Ђакова у Ваљево утицао управо нови ваљевски физикус Јован Сибер. Ова претпоставка је утолико реалнија ако се имају у виду његов неуспели покушај да своју приручну апотеку да граду на употребу без ангажовања апотекара, као и контакти Прикелмајера са члановима породице Сибер у Ђакову, о чему ће даље бити више речи. У сваком случају, Прикелмајер је у Ваљево стигао после Јована Сибера, средином 1969. године. Пошто је са ваљевском општином постигао договор да на годину дана добије бесплатан локал за апотеку, бесплатан стан на спрату изнад ваљевске општине и почетну материјалну потпору и Министарству унутрашњих послова поднео потребна документа, 30. јула 1869. године добио је решење по којем му се „даје дозволење да може отворити и држати апотеку у Ваљеву под условима у закону за апотеке и апотекаре и наставленијима за исте прописаним, са том приметбом да претходно дотичном начелству покаже има ли довољно средства за отварање апотеке и почем спреми апотеку да извести власт да се апотека пре отварања комисијом прегледа" [4]. По добијању овога решења, Клаудије је на кредит, од једне фирме из Пеште, купио сав потребан инвентар и робу [5]. Комисијски преглед, наложен наведеним решењем, обављен је од 3. до 5. септембра, а 9. септембра је Министарству предат извештај по коме је комисија решила да се апотека одмах пусти у јавни рад, с тим што је њеном власнику наложено да исправи све уочене недостатке, који нису били од утицаја за справљање лекова. Тако је прва ваљевска апотека формално почела са радом 5. септембра 1870, али њено фактичко отварање је било знатно раније, чим је из Пеште стигао најосновнији инвентар. Међу документима у породичној архиви породице Прикелмајер чуван је и регистар пазара, по коме је први приход остварен још 27. марта 1870. године и он је износио пет гроша; сутрадан, 28. марта, пазар је био девет гроша и 18 пара, а наредног дана 30 гроша и 16 пара [5].

ВЕЗЕ ПРИКЕЛМАЈЕРА И СИБЕРА

Пошто је фактички почео са радом пре добијања формалне дозволе, Клаудије Прикелмајер се, да би начинио и други животно важан корак, током пролећа 1870. године вратио накратко у Ђаково. У ђаковачкој књизи венчаних уписан је податак да се 15. јуна (по грегоријанском календару) 1870. године „pharm Claudius Josf. Prickalmayer" родом из Пожеге, а настањен у „Valjevo in Serbia" венчао са „Paulina Brauneis" из Ђакова, иначе једном од чланица певачког друштва које је фотографисао 1864. Један од кумова им је био др *Josefus Sieber* [18, 19], по свему судећи најстарији син валповачког др Јосипа, брат ваљевског лекара Јована. Као угледан Ђаковчанин, др Јосип је био кум на венчању многих суграђана, али по свему судећи, ово му је било последње кумство, будући да је умро непун месец и по касније – 28. јула 1870 (по грегоријанском календару) [20].

Са Паулининим доласком у Ваљево 1870. мала ђаковачка колонија у вароши на Колубари је увећана и имала је пет представника. Поред Јована Сибера и Клаудија Прикелмајера, још током 1869. године у Ваљево је из Ђакова стигла и још једна од девојака са Клаудијевих фотографија, Ема Сибер. Она је била Јованова братаница, ћерка Клаудијевог венчаног кума др Јосипа. А како смо већ видели, са Емом или за њом (?) у Ваљево је стигао и Ђуро Козарац. На основу информација из Молбе за отварање приватне гимназије

од септембра 1969, а потом и Молбе за запослење у државну гимназију у оснивању, које су упућене Министарству просвете и црквених дела у априлу 1870. године, Козарац је у Ваљево дошао почетком августа 1869 [21]. Пошто су претходно обоје прешли у православну веру, Ђуро и Ема су се 2. октобра 1869. венчали у ваљевској цркви [22].

Данас је тешко утврдити да ли су Ема и Ђуро заиста стигли истовремено у Београд, па Ваљево, како је то пре једнога века забележио Љубомир Павловић, или је истина нешто другачија. Реална је и претпоставка да је отац др Јосип своју ћерку Ему послао далеко од Ђакова, у Ваљево, код свога брата Јована, да је склони од афере која је избила због недозвољене љубави са католичким свештеником Ђуром, а да је он кренуо за њом. Сумњу у делимичну непоузданост хроничарских бележака буди више нетачних чињеница које Павловић износи: да су се Ема и Ђура заволели у Пожези (*sic!*), где је њен отац Павле (*sic!*) био апотекар (*sic!*), а да је у Ваљеву Ему прихватио стриц Стеван (*sic!*) [6]. Помињање Пожеге, уместо Ђакова, вероватно је условљено сазнањем да је Клаудије Прикеламајер родом из тог славонског места, и стари хроничари су погрешно и друга догађања повезана за Прикелмајера везивали за његово родно место, не знајући да је своју апотекарску каријеру започео у Ђакову и да су многа даља дешавања везана за место његовог рада, а не место рођења. Тако су и фотографије ђаковачког певачког друштва, на којима се налазе и Клаудије и његова будућа супруга Паулина, као и Ема, погрешно потписане: „Аматерска позоришна трупа из Славонске Пожеге“ [4]. А да је Ема неоспорно ћерка ђаковачког доктора Јосипа, а не некаквог пожешког апотекара Павла, сведоче и записи из књиге крштених у Ђакову 1842 [23], као и из књиге венчаних у Ваљеву [22]. А што се тиче помињања имена Стеван, то отвара потребу за додатним истраживањима.

ДОКТОР СТЕВАН СИБЕР

Доктор Јосип Сибер из Ђакова је имао петоро деце – ћерке Ему (1842–1919), Марију (1855–1945) и Густу (1852/3–1936) и синове Јосипа (1844–1920) и Стевана (1848–1903) [10]. Стеван Сибер (Слика 2) заиста је дошао у Србију, у ваљевски крај, али касније, пошто се његова сестра Ема већ удала за Ђуру. Он се у ваљевском крају задржао знатно дуже од стрица Јована и оставио је више трага о свом деловању, тако да је његово име било познатије од Јовановог, са којим је замењен у сећањима хроничара.

Као и његов деда, отац и стричеви, и Стеван је био лекар. Из његове кондуит листе се види да је у Србију дошао 24. јула 1872. године, када је имао 24 године, по свему судећи непосредно по завршеним студијама у Бечу [24]. У то време је његов зет Ђуро био професор ваљевске гимназије, а стриц Јован активни физикус и управник болнице у Ваљеву, тако да су вероватно посредовали да Стеван добије место лекара Тамнавског среза Ваљевског округа, са седиштем у Убу. Током српско-турских ратова 1876–78. био је шеф резервне болнице и апотекар у Лозници, а потом командир санитетског одељења Ваљевске бригаде, лоциране прво на Јавору, па у Ужицу и Чачку. Маја 1874. године његово име је уписано међу лицима која су новчано помогла убско читалиште, а у августу 1884. даровао је аков ракије војницама Петог пука Краља Милана, који су коначили у Убу [3]. Касније, у Српско-бугарском рату 1885. године са чином резервног капетана 1. класе учествовао је у бици на Сливници, у саставу санитетске чете Дринске дивизије и због исказане храбрости и пожртвовања наредне 1886. године одликован је орденом „Таковски крст“ петога реда са мачевима [25]. Те године је, после четрнаест година службовања у Убу, напустио ваљевски крај. Једно време је радио као срески лекар у Зајечару, потом као окружни физикус у Јагодини, Краљеву и Параћину. Пензионисан је 1901. године [26], после чега је наставио приватну праксу у Алексинцу, где је и умро 17. новембра 1903 [10].

Слика 2. Стеван Сибер, у средњим годинама (власништво др Ђорђа Сибера, Београд)
Figure 2. Stevan Sieber as a middle-aged man (property of Dr. Đorđe Sieber, Belgrade)

Расположиви подаци о Сиберима у Србији потврђују изнету тезу о асимилацији досељеника у нову средину. Забележено је да је 11. августа 1882. др Стеван положио заклетву у „Српско сажитељство“, при чему су држављанство Србије добиле и његова супруга Адела и ћерка Јелена [3], а према породичним сећањима после тога је преузео славу Светога Стевана [10]. Са Аделом, ћерком осијечког трговца Фулера, Стеван је

имао више деце. Један од синова, а који је наставио лекарски позив, био је др Ђорђе Сибер (1885–1968). Он је рођен у Убу, гимназију је учио у Крагујевцу и Београду, а медицину је, као војни питомац, студирао у Грацу од 1905. до 1911. године. У чин санитетског поручника је произведен 1912. Током балканских ратова 1912/13. био је лекар Моравске бригаде, затим Приморског одреда деташованог у Албанију и сталне војне болнице у Скопљу. Потом, 1913/14. био је гарнизони лекар у Новом Пазару и Урошевцу. Током Великог рата прво је био лекар при Шумадијској, а на Солунском фронту при Моравској дивизији. Из ратова је изашао у чину мајора. После ослобођења био је управник војне болнице у Суботици, па потом у Београду у краљевој гарди. Затим је радио у Санитетском одељењу Министарства војске и морнарице и као референт санитета II Армијске области у Сарајеву. Од марта 1939. године је бригадни генерал и управник Војнохигијенског завода у Београду, где је 1942. пензионисан од стране владе Милана Недића. После рата се у Београду бавио приватном праксом [10, 27, 28]. Његов син, такође доктор, Ђорђе Сибер (1930–2017), специјалиста грудне хирургије, оснивач и први управник Бронхолошког и ангиолошког одсека Хируршког одељења Института за туберкулозу и један од утемељивача Грудног хируршког центра у Киренаики (Либија), до своје недавне смрти живео је у Београду, и пуно нам је помогао у припреми овога рада. [10].

ЗАКЉУЧАК

Апотекар Клаудије Прикелмајер (1837–1913) и патрон хирургије Јован Сибер (1819–1875) имали су веома запажену улогу у развоју здравствене службе у Ваљеву. Први као оснивач и власник прве ваљевске апотеке, а други као први сталнији управник тек основане болнице. И један и други су у Србију дошли из Славоније, која је тада била део Хабзбуршке монархије, као представници својеврсне образоване економске емиграције која, прелазећи из развијене државе са јаком конкуренцијом у државу у развоју без довољно образованих кадрова, проналази начин за успешан развој каријере. Јован Сибер је у Србију стигао 1852. године, а у Ваљево крајем 1868. или почетком 1869. Иако на основу нижег медицинског образовања, са статусом патрона хирургије, није формално могао да добије радно место окружног физикуса и управника тек основане ваљевске болнице, он је, због недостатка кадрова са вишим образовањем, ипак распоређен на ту функцију. Како је у то време у Ваљеву била актуелна потрага за фармацеутом који би отворио прву апотеку, по свему судећи је управо Јован имао значајну улогу да средином 1869. године у варош на Колубари дође и млади магистар фармације Клаудије Прикелмајер, који је као почетник радио у туђој апотеци у Ђакову, где се дружио са Емом, ћерком Јовановог брата др Јосипа Сибера, који ће му у мају 1870. године бити и кум на венчању. Убрзо, у лето 1869, склањајући се од срамоте изазване љубавном афером, код Јована у Ваљево долази и Ема, а за њом (или са њом) и изабраник њеног срца, католички свештеник Ђуро Козарац. Да је Србија тога времена била погодно тло за развој каријере указује и чињеница да у ваљевски крај 1872. године долази и Емин брат, тек дипломирани др Стеван Сибер (1848–1903), запосливши се као лекар Тамнавског среза, дела Ваљевског округа са седиштем у Убу. Др Стеван је био отац санитетског генерала војске Краљевине Југославије др Ђорђа Сибера (1885–1968), а деда београдског грудног хирурга, такође доктора, Ђорђа Сибера (1930–2017).

НАПОМЕНА

Рад је настао као један од резултата програмске активности Народног музеја Ваљева на проучавању историјата здравства у ваљевском крају, а поводом обележавања 150 година од оснивања Ваљевске болнице (1867–2017).

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Valjevo's medical doctor Jovan Siber and pharmacist Klaudije Prikelmajer – A historical illustration of the role of immigrants from Slavonia in the development of health care in Serbia

Vladimir Krivošejev

National Museum of Valjevo, Serbia

SUMMARY

In a short period of the late 1860s, three significant new institutions were founded in Valjevo: the hospital (1867), the grammar school (1869) and the pharmacy (1870). In the early stages of development of all three institutions, a big role was played by immigrants from Slavonia: medical doctor Jovan Sieber, pharmacist Klaudije Prikelmajer, and professor Đuro Kozarac.

This paper aims to clarify the connections between these individuals that caused their relocation from the territories of the Habsburg Monarchy to Serbia and Valjevo, as well as to resolve the confusion caused by imprecise family memories and written chronicles that have been collected. Moreover, this paper additionally focuses on the origins of the Sieber family, which included Jovan Sieber, and the later arrival of other members of the family to Valjevo. Another member of the family was Jovan's nephew Dr. Stevan Sieber, father of Dr. Đorđe Sieber, who went on to become a general in the Medical Corps of the Royal Yugoslav Army.

Keywords: history of medicine; 19th century; medicine; surgery; Serbia

DOI: https://doi.org/10.2298/SARH170503111I
UDC: 616.411-003.4-089

IN MEMORIAM

Bogdan Bošković (1931–2017)

Bogdan Bošković je rođen 17. septembra 1931. godine u malom selu Konjuh smeštenom dvadesetak kilometara severozapadno od Kruševca, Rasinski okrug. Zahvaljujući svojoj sposobnosti, upornosti, kreativnosti, dobrim učiteljima i pogodnim radnim uslovima uspeo je da završi dva fakulteta (farmaceutski i medicinski), odbrani doktorsku disertaciju, postane vrhunski stručnjak, izuzetno plodan naučnik-toksikolog i veliki učitelj mladim istraživačima.

Praktično ne prestajući da radi, umro je 5. aprila 2017.

Cilj ovog članka je da se prikaže razvoj tog talentovanog stručnjaka, njegov lik i veliki naučno-edukativan doprinos.

Od seoskog dečaka do naučnika

Bogdan je u vreme Drugog svetskog rata sticao osnovno obrazovanje, a zatim je u oslobođenoj zemlji pohađao gimnaziju (Kruševac, 1946–1950). Od 1950. godine je kao vojni stipendista studirao farmaciju na Univerzitetu u Zagrebu. Po završetku studija, 1956. godine, raspoređen je u sanitetsku vojnu službu, a dve godine kasnije postao je specijalizant iz toksikologije bojnih otrova i radio u Vojnosanitetskom zavodu (VSZ, Sarajevo), gde je sarađivao u istraživanjima s poznatim profesorom farmakologije Pavao Šternom. Uskoro potom Bošković postaje student medicine uz rad. (Medicinski fakultet u Sarajevu je u to vreme podsticao asistente raznih predmeta, ako nisu lekari, da studiraju medicinu. Tako se i saradnik profesora Šterna odlučio za dodatno studiranje.)

Vredna je opisa akademska atmosfera na Institutu za farmakologiju koju je formirao profesor Štern. Kada je posle rata, 1946. godine, trebalo obnoviti u ratnim godinama osnovan Medicinski fakultet u Sarajevu, ukazom vlade FNRJ mladi nastavnici iz Zagreba i Beograda su prešli u Sarajevo, praktično na sve predmete. Profesor Štern je bio jedan od pridošlica. On je već bio istaknuti farmakolog-istraživač, koji je u Zagrebu usko sarađivao s hemičarem Vladimirom Prelogom (rođenom u Sarajevu), koji je kasnije, na poziv nobelovca Leopolda Ružičke otišao u Švajcarsku, gde se stručno afirmisao i 1975. godine dobio Nobelovu nagradu. Pod vođstvom profesora Šterna u Sarajevu se razvila farmakološka škola, u kojoj su radili zaposleni lekari, biohemičari i biolozi, kao i mnogi saradnici s drugih instituta ili katedri Medicinskog fakulteta i Univerziteta u Sarajevu i gostujući istraživači iz mnogih gradova Jugoslavije i inostranstva. Da bi istraživanja Šternovih saradnika bila što uspešnija, on je svoje asistente podsticao da odlaze na duže usavršavanje u poznate svetske laboratorije. Tako je Seid Huković proveo dve godine u Engleskoj i jednu u Zapadnoj Nemačkoj, Dobrila Ratković i Dubravka Potkonjak po dve godine u Engleskoj i Rajko Igić dve godine u SAD. Ime profesora Šterna je zabeleženo među pronalazačima antihistaminika (*Kuschinsky G, Lüllmann H, Mohr K. Kurzes Lerbuch der Pharmakologie und Toxicologie. Stuttgart, Georg Thieme Verlag*, 1993), a i po tome što je bio organizator Prvog simpozijuma o supstanci P (Slika 1). Na tom simpozijumu je učestvovao i njegov naučni saradnik Bogdan Bošković.

Sposoban, vredan i savršeno organizovan, mladi Bogdan Bošković imao je u sarajevskom miljeu podsticaje koji su omogućili da sa saradnicima publikuje brojne istraživačke radove iz farmakologije i toksikologije. Sa profesorom Šternom je, prema bazi podataka *Medline*, objavio 14 radova u domaćim i stranim časopisima, a Boškovićevi koautori, pored Šterna, iz sarajevskog perioda su M. Deželić, B. Nikolin, S. Hadžović, H. Krnjević, D. Potkonjak, Z. Popović, E. Bašagić, R. Pržić i M. Ciglar. Već broj ovih imena ukazuje na široku saradnju sa istraživačima koju će Bogdan odlaskom u Beograd toliko proširiti da je u njegovoj generaciji teško mu naći para, bilo gde u našoj (bivšoj i sadašnjoj) zemlji.

Примљено • Received: May 3, 2017
Прихваћено • Accepted: May 5, 2017
Online first: May 9, 2017

Slika 1. Fotografija učesnika Prvog simpozijuma o supstanci P, na kojoj je i Bogdan Bošković. Učesnici su bili naučna elita tog doba: Marta Fogt, G. Cetler, D. Beleslin, V. Varagić, B. Pernau, K. Lisak, S. Huković, F. Lembek, B. Radmanović, M. Milošević, U. S. fon Ojler (stoji treći s desne strane), P. Štern (stoji šesti s desne strane). Treći sa leve strane stoji Bogdan Bošković. Ovaj (jedno vreme zagubljen) snimak načinio je ser Džon Henri Gadam, koji je zajedno s Ulfom Svanteom fon Ojlerom otkrio supstancu P, a Pavao Štern je sa svojom ekipom vodio veoma važna istraživanja o toj novootkrivenoj supstanci. Fon Ojler je 1970. godine dobio Nobelovu nagradu za otkriće da je noradrenalin neurotransmiter u adrenergičkom nervnom sistemu. Fon Ojlerovo učešće na simpozijumu u Sarajevu olakšalo je njegove posete Beogradu (1968) i Tuzli (1982).

Rad u Vojnotehničkom institutu i Vojnomedicinskoj akademiji u Beogradu

U jesen 1963. godine bio sam student u prvoj generaciji postdiplomaca na Medicinskom fakulteta u Sarajevu. Te studije je organizivao i vodio profesor Štern. Tada sam upoznao Bogdana Boškovića, koji je povremeno dolazio na Institut za farmakologiju, gde bi se kratko zadržavao, samo da obavi nužne dogovore. Uvek je bio vedar, dobro raspoložen i sa svima ljubazan. Naredne godine je odbranio doktorsku disertaciju, a 1968. godine je završio studije medicine; u to vreme sam bio asistent na predmetu Farmakologija i toksikologija. Njegov definitivni odlazak bio je za sve na Institutu za farmakologiju gubitak, ali je prelazak u Beograd bila velika prilika i podsticaj za njegove nove izazove.

Bošković je 1964. i 1969. godine bio na šestomesečnim usavršavanjima iz farmakologije u Engleskoj (Kembridž i London). U Beogradu je radio u Vojnotehničkom institutu (VTI), gde su pod njegovim rukovodstvom istraživani mehanizami dejstva bojnih otrova (cijanidi, plikavci, nervni otrovi) i traženi efikasni antidoti i sredstava za zašitu od tih otrova. Takođe su ispitivane mogućnosti zaštite od štetnih uticaja raznih bioloških agenasa, uključujući pronalaženje hemijskih radioprotektora kojima se sprečava ili ublažava radijaciona bolest. Rezultate tih istraživanja Bošković je sa saradnicima publikovao u eminentnim međunarodnim časopisima, što je dopinelo da se naša vojna toksikologija nađe u svetskom vrhu. Zato se među vojnim toksikolozima na međunarodnim stručnim skupovima često čula reč o Beogradskoj toksikološkoj školi. Bogdan Bošković je objavio ukupno više od 400 naučnih i stručnih radova, a važnije publikacije su mu citirane preko 750 puta. Najčešće je citiran njegov rad „Prophylaxis and Treatment of PAM-2 Cl, HI-6, and HGG-12 in soman and tabun poisoning“ (112 puta), publikovan u *Fundamental and Applied Toxicology* (1984; 4:S106–15).

U SAD, Velikoj Britaniji i na šest međunarodnih skupova iz toksikologije i farmakologije Bošković je držao predavanja po pozivu. Bio je član Srpskog lekarskog društva i društava farmakologa, toksikologa i farmaceuta u zemlji. U 1971. godini izabran je za člana Britanskog farmakološkog društva, a 1996. godine za člana Nemačkog društva za farmakologiju i toksikologiju. U vojsci bivše Jugoslavije obavljao je važne funkcije u Savetu za naučni rad, a bio je član i predsednik komisije za naučnoistraživački rad nastavno-naučnog veća Vojnomedicinske akademije (VMA). U vojnoj hijerarhiji je od sanitetskog kapetana dosegao čin pukovnika.

Nastavnik i mentor

Na VMA, Bošković je na predmetu Farmakologija biran u zvanje docent (1972. godine), vanredni profesor (1976. godine) i redovni profesor (1981. godine). Na Medicinskom fakultetu u Tuzli bio je od 1986. do 1990. godine profesor na poslediplomskim studijama, a od 1998. godine predavao je (kao gostujući profesor) farmakologiju i toksikologiju na Medicinskom fakultetu u Foči. On nije svoju istraživačku aktivnost i nastavnički rad usmeravao samo na kadrove VTI i VMA, naučna istraživanja je obavljao s velikim brojem istraživača širom zemlje. Tako je u Tuzli podstakao istraživanje iz svoje oblasti i ti su rezultati kasnije publikovani (*Effect of soman and quinalphos on diuresis and urinary kallikrein excretion. Yugoslav Physiol Pharmacol Acta* 1991; 26:315–9). Pored toga, u Tuzli je intenzivno sarađivao s jednim tadašnjim postdiplomcem. Celokupna Boškovićeva edukativna aktivnost jasno se uočava iz ovih jednostavnih statističkih podataka: bio je mentor ili komentor u 68 doktorskih disertacija i u 52 magistarska rada. Pored toga, sarađivao je u istraživanjima s mladim kolegama širom zemlje.

Epilog

Kada procenjujemo najvažniji doprinos profesora Boškovića, mogli bismo tvrditi da je njegova edukativna delatnost najvažnija. On je doprineo širenju i razvoju naučnih istraživanja u našim krajevima, uključujući Beograd, Novi Sad, Zagreb, Niš, Sarajevo, Tuzlu, Rijeku i Prištinu. Njegovo celokupno delo i široka naučna saradnja podizali su naš ugled u svetu, koji nam nije uvek naklonjen. Bošković je, zajedno s nekolicinom drugih đaka profesora Šterna, nastavio delo tog velikog istraživača i učitelja, ali i drugih naših velikana, poput Milana Jovanovića-Batuta, Ivana Đaje, Milutina Milankovića, Andrije Štampara, Draga Perovića, Branka Šljivića, Aleksandra Sabovljeva, Nikše Algretija, Jelene Krmpotić-Nemanić, Grujice Žarkovića i drugih. Neki Boškovićevi đaci su već pošli stopama svog učitelja.

Vredi istaći važnost širenja nauke i kvalitetnog visokog školstva u zemljama koje su zbog specijalnih istorijskih razloga bile u zaostatku, da bi se velikim koracima išlo napred. Detalji o savetima profesora Šterna i drugih naučnika mogu se naći u tekstu posvećenom mladim istraživačima koji je objavio časopis *Galaksija nova* (http://galaksijanova.rs/mladom-istrazivacu/). Međutim, ne mogu samo vrhunski istraživači svojim ugledom, znanjem, radom i dostignućima doprinositi razvoju nauke u zemlji. Političari i privrednici koji vode zemlju treba da shvate važnost bazičnih i primenjenih nauka za napredak zemlje i da materijalno podstiču razvoj te delatnosti. Udruženim snagama naučnika i društvene zajednice dolazi do prosperiteta zemalja koje ne poseduju obilje prirodnih bogatstava (nafta, gas, rude i drugi prirodni izvori). Ako nam razvoj nauke postane primarni cilj, jednog dana ćemo ličiti na Švajcarsku. Bogdan Bošković je dao svoj doprinos.

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DOI: https://doi.org/10.2298/SARH170503110J
UDC: 61(091)

ПИСМО УРЕДНИКУ / LETTER TO THE EDITOR

Историја медицине: у чему је проблем

Поштовани уреднице,

Радује ме што су у Српском архиву објављена два веома квалитетна рада из историје медицине. Они се могу посматрати и као репрезенти два основна правца у изучавању ове научне области. Први рад – *Прва планирана ресекција јетре у Србији* [1] из области је историје медицине у ужем значењу (историја медицинске теорије и праксе). Други, под насловом *Ваљевски лекар Јован Сибер и апотекар Клаудије Прикелмајер – историјска скица о улози досељеника из Славоније на развој здравства у Србији* [2], из области је историје здравствене културе, неодвојиве од историјског, општег културног, социјалног, политичког и економског контекста развоја једне друштвене заједнице. Радови долазе „у добри час", како је говорио Јован Данић (1854–1924), дугогодишњи уредник *Српског архива* и *Народног здравља*, и као основа за разматрање појединих проблема у области историје медицине који се тичу њене прошлости, садашњости и будућности.

Од свог утемељења као академске дисциплине (прво у Немачкој, у 18. веку), историја медицине је често била (и још увек јесте) предмет критичких разматрања у погледу сврсисходности (првенствено у курикулумима студија медицинских наука), праваца истраживања и тумачења, и, коначно, „припадности" (медицинским или хуманистичким и друштвеним наукама, односно професионалцима у истим областима). Ако би, у најширој периодизацији, њена институционализација представљала прву фазу развоја, следећа би била означена појавом нове парадигме – друштвене историје медицине, коју је тридесетих година 20. века промовисао Хенри Сигерист (*Henry E. Sigerist*, 1891–1957). Сигерист је историју медицине, до тада традиционално „лекарску" област, повезао са друштвеном историјом и ставио је у функцију социјалне медицине [3], истичући да историја медицине није само „историја великих људи и великих идеја" већ и „историја човека у здрављу и болести". „Ми знамо много о историји великих медицинских открића", истицао је, „али знамо врло мало о томе да ли су и на кога она била примењена" [4]. Указивао је, такође, на потребу повезивања историјско-медицинских тема са појавама које утичу на људско здравље у савременом добу. Сигеристове смернице, које су у прво време прихватили малобројни следбеници, заживеле су у већој мери тек седамдесетих и осамдесетих година, и то углавном у земљама западног света. Ширење истраживачког оквира иницирало је ширење научних заједница које су почеле да окупљају стручњаке различитих професија – историчаре, социологе, антропологе, демографе, филозофе, филологе... Од почетне мултидисциплинарности, размене идеја, знања и искустава водиле су ка усвајању интердисциплинарног приступа у проучавању проблема.

Изучавање историје медицине у Србији има традицију дугу готово један и по век. По реду оснивања, први „центар" за историју медицине био је Медико-историјски музеј Централног хигијенског завода (1937–1945/46); други је била Секција за историју медицине СЛД (1950); трећи – Секција за историју медицине, фармације и ветеринарства САН (1955–1961); четврти – Југословенско друштво за историју медицине, фармације и ветерине (1955, преименовано 1962. године у Научно друштво за историју здравствене културе Југославије, а 1977. у Савез научних друштава за историју здравствене културе Југославије. Секције овог друштва – Секција САП Војводине (1964) и Секција СР Србије (1965), постале су 1977. научна друштва у оквиру савезног – Научно друштво за историју здравствене културе Србије (активно до 1991) и Научно друштво за историју здравствене културе Војводине (активно и данас).

Изучавање историје медицине је бележило успоне у периодима организованог рада, посебно у другој половини 20. века, када је најшири научни форум било Научно друштво за историју здравствене културе Југославије. Поред медицинских професионалаца, у Друштву су били активни историчари, етнолози, археолози, филолози и библиотекари, па су истраживачке теме

Примљено • Received: May 3, 2017
Прихваћено • Accepted: May 5, 2017
Online first: May 9, 2017

биле разнолике. Оне су, међутим, у већој мери остале традиционалне (фокусиране на биографије великана и развој здравствених установа). Средином шездесетих година, о проблемима изучавања историје медицине у Југославији писао је Зденко Левнтал (1914–1999), професор историје медицине на Медицинском факултету у Београду (1959–1969), касније на Универзитету у Берну. Он је у Србији био можда једини следбеник Сигеристовог модернизма. Залагао се за оснивање Института за историју медицине, за ширење оквира истраживачких тема и њихово разматрање у контексту националне и светске историје и културе. Истицао је да би дотадашњи „само коегзистентни рад лекара, фармацеута, ветеринара и других" у југословенском научном друштву требало да постане „интеграциони и синхронизован" [5].

Шта се променило за педесет година, колико је прошло од Левнталових критика и смерница? Од три научна друштва за историју здравствене културе – југословенског, српског и војвођанског, прва два су се угасила с распадом СФРЈ; Секција за историју медицине СЛД, неактивна од 1991. године, с великим успехом обновила је рад 2009. и поново окупља истраживаче различитих образовних профила (добитник је Награде за најбољу секцију СЛД у 2016); историја медицине је изборни предмет на основним студијама на медицинским факултетима у Новом Саду (од 1962) и у Београду (поново од 2004/05). Али оно што би требало да буде заступљеније јесу „нови" приступи изучавању медицинско-историјских тема (сагледавање и анализирање појава и проблема у контексту односа медицина – здравље – друштво) и ширење тематског оквира (искуства болесника; однос лекар – болесник и многе друге). Унапређењу дисциплине кључно би допринели професионализација (остварива кроз интердисциплинарно последипломско образовање у овој области) и институционализација (установљење института за историју медицине или историју наука и медицине) као предуслови стварања школе историје медицине.

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Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонораришу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални (научни и стручни) радови, метаанализе, прегледни радови, претходна и кратка саопштења, прикази болесника и случајева, слике из клиничке медицине, видео-чланци, радови за праксу, актуелне теме, радови из историје медицине и језика медицине, лични ставови, наручени коментари, писма уреднику, прикази књига и други прилози. Оригинални радови, претходна и кратка саопштења и прикази болесника и случајева публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}*Tc*, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало

би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, метаанализу, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење, метаанализе и прегледне радове, сажетак треба да има следећу структуру: Увод/Циљ, Методе, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh)*.

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом.

Уколико је рад у целости на српском језику (нпр. рад из историје медицине, језика медицине и др.), потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик. Сажетке и радове који су у целости на српском језику аутори из Србије треба да пишу ћирилицом.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад, метаанализа, претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор метаанализе и прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада – који чине насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, претходно и кратко саопштење, рад из

историје медицине и преглед литературе до 5.000 речи, а за приказ болесника, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3.000 речи; радови за остале рубрике могу имати највише 1.500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

ТАБЕЛЕ. Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле.

Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

СЛИКЕ. Слике су сви облици графичких прилога и као „слике“ у СА се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *е*-издању и објављена у штампаном издању.
Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

ГРАФИКОНИ. Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета.

Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

СХЕМЕ (ЦРТЕЖИ). Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме.

Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, а у метаанализи до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да

буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примери навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) за годину у којој се рад предаје Уредништву. Сви домаћи аутори такође морају бити претплаћени на часопис или измирити накнаду за обраду чланака (*article processing charge*) за годину у којој се рад предаје Уредништву, у износу од 3.000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*article processing charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе. Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

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ISSN 0370-8179
ISSN Online 2406-0895

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ISSN 0370-8179
ISSN Online 2406-0895

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61(497.11)

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Двомесечно. - Ima suplement ili prilog: Српски архив за целокупно лекарство. Суплемент = ISSN 0354-2793. - Drugo izdanje na drugom medijumu: Српски архив за целокупно лекарство (Online) = ISSN 2406-0895
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