YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2019; July Vol. 76 (No. 7): p. 663–756.

Vojnosanitetski Pregled 2019 July Vol. 76 (No. 7): p. 663–756.

vol. 76, br. 7, 2019

VOJNOSANITETSKI PREGLED

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

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

IZDAVAČ
Univerzitet odbrane, MO Republike Srbije

ISSN 0042-8450
eISSN 2406-0720
Open Access
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Adresa redakcije: Univerzitet odbrane, Institut za naučne informacije, Crnotravska 17, 11 040 Beograd, Srbija.
Informacije o pretplati: Tel.: +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

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

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-19540845-28, poziv na broj 122742313338117. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. +381 11 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa Vojna štamparija, Beograd, Resavska 40b

vol. 76, No. 7, 2019

VOJNOSANITETSKI PREGLED

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

PUBLISHER
University of Defence, Ministry of Defence of the Republic of Serbia, Belgrade, Serbia

ISSN 0042-8450
eISSN 2406-0720
Open Access
(CC BY-SA)

Editorial Office: University of Defence, Institute for Scientific Information, Crnotravska 17, 11 040 Belgrade, Serbia.
E-mail: **vsp@vma.mod.gov.rs**

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

The Journal is published monthly. Subscription: Giro Account No. 840-19540845-28, refer to number 122742313338117. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40b

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ORIGINAL ARTICLES / ORIGINALNI RADOVI

Cadets of the Faculty of Medicine of the Military Medical Academy of the University of Defence in Belgrade, future military physicians, at an exercise where they demonstrated their practical knowledge and skills in the care of the injured.
This year, July 30th, 180 years have passed since the organized medical service in the Serbian Army was established. That day is celebrated as the Day of the Serbian Army Medical Service.

Kadeti Medicinskog fakulteta Vojnomedicinske akademije Univerziteta odbrane u Beogradu, budući vojni lekari, na vežbi na kojoj su pokazali svoja praktična znanja i veštine u zbrinjavanju povređenih.
Ove godine, 30. jula, navršava se 180 godina od ustanovljenja organizovane sanitetske službe u Vojsci Srbije. Taj datum slavi se kao Dan Sanitetske službe Vojske Srbije.

ORIGINAL ARTICLES

UDC: 616.89-008.441.44-02:364.63-053.2
https://doi.org/10.2298/VSP170826142D

Victimization in childhood as a suicide risk factor in adults

Viktimizacija u detinjstvu kao faktor suicidnog rizika kod odraslih

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Abstract

Background/Aim. There is a burgeoning literature on the association between childhood victimization and the risk of suicidal behavior in early adolescence, while there is significantly less research showing this association in adults. The aim of our study was to examine whether victimization in childhood increased the likelihood of suicide attempt in adults. **Methods.** The sample consisted of 90 patients, 71 females and 19 males, aged 37.92 ± 11.04 years on average, hospitalized in the Day Hospital of the Clinic of Psychiatry Military Medical Academy, Belgrade, Serbia. The Juvenile Victimization Questionnaire (JVQ), Defense Style Questionnaire (DSQ-40) and Beck Depression Inventory were used for 50 patients following suicide attempt and in 40 patients who were on psychotherapeutic treatment due to various life crises not resulting in suicide attempt. According to the indications, we excluded the patients with psychosis (F20-F29, F30-31 and F 32.3), substances abuse (F10-F19) and dementia (F00-F09), satisfying International Classification of Diseases-10 version (ICD-10) (the World Health Organization criteria). The examinees of both groups were matched by age, education and marital status. Comparison of the patient groups was done by the Students' *t*-test for the parametric features and Mann-Whitney U test for non-parametric data. **Results**. The suicide attempters had moderate depression (19.76 ± 10.52) and used immature defense mechanisms ($p < 0.001$). The JVQ established statistical differences in the Total score ($p < 0.005$) and in two modules: Peer and Sibling Victimization ($p < 0.005$) and Sexual victimization ($p < 0.005$). **Conclusion**. The adults who were more likely to attempt suicide during their lifetime were more often victims of peer and sexual abuse in their childhood. Data on victimization in early childhood provide opportunities for early detection of persons with suicide risk that could help in the psychotherapeutic work with these patients, but also in the suicide prevention in a wider population.

Key words:
crime victims; child; suicide, attempted; adults; psychotherapy.

Apstrakt

Uvod/Cilj. Postoje istraživanja o povezanosti između viktimizacije deteta i rizika od samoubilačkog ponašanja u ranoj adolescenciji, dok je značajno manje istraživanja koja pokazuju ovu povezanost kod odraslih osoba. Cilj našeg istraživanja je bio da se ispita da li viktimizacija u detinjstvu povećava rizik od pokušaja suicida kod odraslih osoba. **Metode.** Istraživanje je sprovedeno na grupi od 90 pacijenata, 71 žene i 19 muškaraca, prosečne životne dobi od 37,92 ± 11,04 godina, hospitalizovanih u Dnevnoj bolnici Klinike za psihijatriju Vojnomedicinske akademije, Beograd, Srbija. U istraživanju su korišteni: Upitnik viktimizacija (*The Juvenile Victimization Questionnaire* – JVQ), Upitnik mehanizama odbrane (*Defense Style Questionnaire* – DSQ-40) i Bekova skala depresije (*Beck Depression Inventory* – BDI). Ispitanici su bili podeljeni u dve grupe: grupa pacijenata koji su pokušali suicid (N = 50) i grupa pacijenata koji su bili na psihoterapijskom tretmanu zbog različitih životnih kriza koje nisu imale za posledicu pokušaj samoubistva (N = 40). Iz ispitivanja su isključeni pacijenti sa psihotičnim poremećajem (F20-F29, F30-31 i F 32.3), bolestima zavisnosti (F10-F19) i demencijom (F00-F09), prema Međunarodnoj klasifikaciji bolesti 10 verzija (ICD 10) (*World Health Organization criteria*). Ispitanici obe grupe su bili ujednačeni prema godinama života, obrazovanju i bračnom statusu. Za statističku obradu korišten je Studentov *t*-test za parametarsku i Mann-Whitney U test za neparametarsku analizu podataka. **Rezultati**. Pacijenti koji su pokušali suicid ispoljavali su depresiju umerenog stepena (19.76 ± 10.52) i koristili su nezrele mehanizme odbrane ($p < 0.001$). Upitnikom JVQ utvrđene su statistički značajne razlike na Ukupnom skoru viktimizacije ($p < 0.005$), kao i na dva modula: Vršnjačko zlostavljanje ($p < 0.005$) i Seksualno zlostavljanje ($p < 0.005$). **Zaključak.** Odrasle osobe koje su

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češće tokom života pokušale samoubistvo su bile žrtve vršnjačkog i seksualnog zlostavljanja u detinjstvu. Podaci o zlostavljanju u ranom detinjstvu pružaju mogućnosti rane detekcije osoba sa suicidnim rizikom što može pomoći u psihoterapijskom radu sa ovim osobama, kao i u prevenciji suicida u široj populaciji.

Ključne reči:
žrtve zločina; deca; samoubistvo, pokušaj; odrasle osobe; psihoterapija.

Introduction

Suicide risk factors are estimated on the basis of the presence or absence of a number of specific factors that are presumed to be significant in order to have a suicide occurring within the statistical probability. Bearing in mind all these characteristics and regularity as well as belonging to certain risk groups and knowing the specific life situations in which an individual can be found and attempt suicide, more or less reliable estimates of the degree of risk can be made as well as the basic motives of a person willing to attempt suicide [1].

In recent years, an increasing importance in investigations of the distal sucide risk factors has been given to the problem of child victimization. In prospective family studies of suicide risk monitoring across many generations, some data on the early childhood experiences of physical and sexual abuse and child neglect in the family were encountered, and they represent significant risk factors for suicidal behavior in adolescence [2–5].

Investigations show that the childhood maltreatment is frequent among western societies, with an estimated prevalence of 10% to 15% [2].

Data obtained from the national study among the U.S. adults who attempted suicide showed that the prevalence of reported childhood abuse was 4.60% for physical abuse, 7.83% for emotional abuse and 10.20% for sexual abuse. Approximately 18% of adults reported some form of violent behavior in their childhood [6].

The results from a representative sample of 5,960 students (aged 17) from high schools in Sweden showed that 84.1% (83.0% young men and 85.2% young women) of the students had experienced victimization during their lifetime, and 10.3% were categorized as poly-victims (8.1% young men and 12.5% young women) [7].

The results from the South Australian population-based observational study suggest that there is a strong association between a history of childhood bullying victimization and current suicidal ideation that persists across all ages [8]. In a random probability sample comprising 7461 respondents in Great Britain interviewed on psychiatric morbidity of adults, recalled of being bullied in childhood decreased with age from 25% of 16-24 year old subjects to 4% among those of 75 or over, with few differences in proportions between men and women. Adults who reported bullying in childhood were still more than twice as likely as other adults to attempt suicide later in life [9].

Some investigations estimated differences in gender in childhood abuse. An investigation among Swedish students showed that the adolescents living with both parents were at lower risk of any form of victimization for both genders. But, females living with both parents were at higher risk of maltreatment, peer victimization, and most significantly, sexual victimization [7].

Childhood maltreatement is a risk factor for the development of mental disorders in adulthood [10]. Suicide attempts are more often seen in young people from dysfunctional families characterized by divorce, psychopathology in parenting, data on sexual, physical and emotional abuse or neglect, poor parent-child relationships, quarrels and violent behavior among parents. The problem is even more dramatic if disturbed family relationships last for a long time, with the tendency of accumulation and the occurrence of multiple child abuse, which include socioeconomic problems, alcohol abuse in the family, or frequent relocation.

The data obtained from the British National Child Development Study, from 7,771 participants, a 50-year prospective cohort of births in 1958 suggested that maltreatment in childhood and early adolescence increased levels of psychological distress at ages 23 and 50 [9]. The victims of frequent bullying had higher rates of depression, anxiety disorders and suicidality than their nonvictimized peers. Childhood bullying victimization was associated with a lack of social relationships, economic hardship and perceived quality of life at age 50 as poor [10].

In the individuals hospitalized at the Clinic for Toxicology at the Military Medical Academy (MMA), Belgrade, following suicide attempt by self-poisoning, 86.7% of suicide attempters had a history of bullying just preceding the suicide attempt: 53.3% by their mother or father (emotionally mistreated and/or physically bullied), 23,3% by their conjugal partner (sexually neglected and/or emotionally harassed, or physically bullied), and 10% by persons in their social network (emotionally neglected and/or bullied) [11, 12].

The largest number of studies show that there is an association between childhood abuse (victimization) and the risk of suicidal behavior in early adolescence, while significantly less studies show this association in adulthood. This is one of the reasons for our interest in the relationship between the two types of behavior.

The objective of our study was to determine whether a history of childhood victimization is associated with suicide attempt in adult life.

Methods

The cross-sectional study was performed on a sample consisting of 90 consecutively recruited patients, 71 females and 19 males, who were on psychotherapeutic treatment in the Day Hospital of the Clinic of Psychiatry MMA, Belgrade, Serbia. The investigation was conducted during 3-year period (2014 to 2017.).

The majority of patients were recruited directly after the inpatient treatment in the Clinic of Psychiatry, MMA, where they were admitted after the treatment at the Clinic for Toxicology, MMA, following a suicide attempt by self-poisoning. A few patients admitted after an outpatient examination at the Department of Psychiatry, MMA, and self-reported that they had a lifetime suicide attempt.

The most common reasons for suicide attempt were separation problems and problems with interpersonal communication with emotional partners. The patients with diagnosis F32-F33, F40-F48 were included into the control group. According to the indications, the patients who were excluded from our investigation suffered from: dementia (F00-F09), substance abuse/dependence (F10-F19) and psychotic disorders (F20-F29, F30-31, 32.3) satisfying International Classification Disease-10th version (ICD 10) [13].

This study was conducted according to the approval by the Ethics Committee of the Military Medical Academy Belgrade. The written informed consent after receiving the information about the study was obtained from all patients prior to their inclusion into the study. Confidentiality of the responses was assured.

Patients

The patients were divided into two groups. The first group consisted of 50 suicide attempters (38.76 ± 10.26 years old with 13.51 ± 2.16 years of education). The control group (non-suicide attempters) consisted of 40 patients who were on psychotherapeutic treatment due to various life crises, not resulting in suicide attempt (37.55 ± 11.63 years old with 13.70 ± 2.31 years of education). A half of all patients of both groups were married and had children, and about 20% came from uncomplete primary family. The patients of both groups were matched by age, education, and characteristics of primary and secondary family (marital status, children and primary family completed).

Instruments

The demographic data were collected from medical records.

The following was used in the investigation: The Juvenile Victimization Questionnaire (Adult Retrospective Questionnaire – JVQ) [14], Defense Style Questionnaire (DSQ-40) [15] and Beck Depression Inventory (BDI) [16].

The Juvenile Victimization Questionnaire (Adult Retrospective Questionnaire) is a comprehensive questionnaire designed to gather information for a variety of important forms of victimizations experiences during their childhood, including community violence and other conventional crime, bullying and other peer and sibling violence and witnessing all types of violence, including domestic violence. The JVQ is a self-report questionnaire adapted for the retrospective reporting of childhood events starting from infancy to 17 years of age, which is assessed from the perspective of an adult. It contains 34 types of abuse that adults experienced during their childhood and adolescence. It covers five general areas of concern (modules): Conventional crime, Child maltreatment, Peer and Sibling victimization, Sexual victimization and Witnessing and indirect victimization. Every victimization-screening question includes the number of times a child was victimized, who victimized the child, whether the child was hurt and questions specific to the victimization reported [14]. The Juvenile Victimization Questionnaire is free for public to use. We translated it in Serbian for our investigation.

The Defense Style Questionnaire (DSQ-40) consists of 40 claims of personal attitudes. It includes 20 defense mechanisms; each mechanism is represented by two questions. Mature and the neurotic defence mechanisms include 8 questions and the immature include 24 questions. Using the scale of 9 numbers, each respondent is asked to indicate how much he agree or disagree with the given statements. The defense mechanism score represents the sum of all items of the same set. The score of each defense mechanism is calculated as the average response to items that make this defense mechanism [15].

The Beck Depression Inventory is a scale for assessing depression. There are 21 questions with 4 answer options graduaded from four point Likert scale from 0–3. The total score is the sum of all answers. The cut-off score for clinically significant depression is 10. The higher score indicates more severe depressiveness [16].

Statistical analysis

The one-sample Kolmogorov-Smirnov test was used for the testing the normal distribution of data. *P*-value 0.005 was considered to be significant and *p*-value of 0.001 to be highly significant.

Results

Defense mechanisms (DSQ-40) and Depression BDI are shown in Table 1.

Table 1

Defense mechanisms (DSQ-40) and Depression (BDI) in suicide and non-suicide attempters

Variable	Suicide attempter (n = 50) mean ± SD	Non-suicide attempter (n = 40) mean ± SD	*p*
Depression	19.76 ± 10.52	17.08 ± 11.89	0.470
Defense mechanisms			
neurotic	5.23 ± 1.36	4.82 ± 1.55	0.602
immature	4.80 ± 0.92	4.50 ± 1.44	0.001**
mature	5.62 ± 1.15	5.39 ± 1.43	0.198

DSQ – Defense Style Questionnaire; BDI – Back Depression Inventory; $p < 0.005$*; $p < 0.001$; SD – standard deviation.**

The patients who attempted suicide and the patients from the control group had depression of moderate level, and there were no statistically significant differences between the groups of patients.

The patients who attempted suicide had the higher values of the total score of mature, immature and neurotic defense mechanisms than the patients from the control group. But, there were highly statistically significant differences in the immature defense mechanisms between two groups of patients ($p < 0.001$).

The Juvenile Victimization Questionnaire showed that there was a high statistically significant difference beetween the suicide attempters and the control group in the Total score of the Questionnaire ($p < 0.005$).

In the module related to Peer and Sibling victimization, all values were higher in the suicide attempters than in the control group. There were statistically significant differences in the subscale of Bullying and in the Total score ($p < 0.005$).

In the module of Sexual Victimization all values were higher in the suicide attempters than in the control group. There was a statistically significant difference in the subscale of Statutory Rape & Sexual Misconduct ($p < 0.005$) and in the Total score ($p < 0.005$) (Table 2).

Table 2

Results of Juvenile Victimization Questionnaire (JVQ) in suicide and non-suicide attempters

JVQ	Suicide attempter mean ± SD	Non-suicide attempter mean ± SD	z	*p*
Conventional crime				
robbery	1.24 ± 1.88	0.75 ± 1.48	1,582	0,114
personal theft	0.96 ± 1.37	0.60 ± 0.93	1,208	0,227
vandalism	1.18 ± 1.55	0.90 ± 1.71	1,816	0,069
assault with weapon	1.22 ± 1.93	0.45 ± 0.81	1,705	0,088
assault without weapon	1.76 ± 2.19	1.48 ± 2.04	0,731	0,465
attempted assault	1.20 ± 1.77	0.45 ± 0.99	2,291	0,022
kidnapping	0.20 ± 0.14	0.00 ± 0.00	0,894	0,371
bias attack	0.58 ± 1.49	0.23 ± 1.00	1,842	0,065
total score	8.16 ± 7.78	4.77 ± 5.27	2,277	0,023
Child maltreatment				
physical abuse by caregiver	2.68 ± 2.58	2.23 ± 2.47	0,830	0,407
psychological/emotional abuse	2.22 ± 2.65	1.88 ± 2.52	0,485	0,628
neglect	0.58 ± 1.60	0.18 ± 0.96	1,412	0,158
custodial interference/family abduction	0.32 ± 1.19	0.23 ± 0.97	0,255	0,799
total score	5.80 ± 5.48	4.50 ± 5.35	1,359	0,174
Peer and sibling victimization				
gang or group assault	0.74 ± 1.51	0.35 ± 1.23	1,961	0,050
peer or sibling assault	3.06 ± 2.46	1.88 ± 2.02	2,238	0,025
nonsexual genital assault	0.18 ± 0.52	0.02 ± 0.22	1,421	0,155
bullying	2.16 ± 2.41	0.72 ± 1.50	3,132	0,002*
emotional bullying	1.76 ± 2.48	0.72 ± 1.61	1,484	0,138
dating violence	0.24 ± 0.87	0.17 ± 0.54	0,023	0,981
total score	8.14 ± 6.95	3.97 ± 4.63	3,100	0,002*
Sexual victimization				
sexual assault by known adult	0.28 ± 0.90	0.25 ± 0.77	0,587	0,557
nonspecific sexual assault	0.16 ± 0.42	0.02 ± 0.22	1,421	0,155
sexual assault by peer	0.20 ± 0.83	0.00 ± 0.00	1,819	0,069
rape: attempted or completed	0.42 ± 1.19	0.02 ± 0.26	1,527	0,127
flashing/sexual exposure	0.48 ± 1.26	0.27 ± 1.01	1,929	0,054
verbal sexual harassment	0.96 ± 2.06	0.20 ± 0.96	1,952	0,051
statutory rape & sexual misconduct	0.34 ± 1.11	0.00 ± 0.00	2,633	0,005*
total score	2.84 ± 5.70	0.90 ± 2.79	2,960	0,003*
Witnessing and indirect victimization				
witness to domestic violence	1.06 ± 1.82	0.97 ± 1.79	0,590	0,555
witness to parent assault of sibling	0.78 ± 1.86	0.02 ± 0.34	2,131	0,033
witness to assault with weapon	1.34 ± 1.99	0.67 ± 1.59	1,822	0,068
witness to assault without weapon	1.64 ± 2.04	1.12 ± 1.96	1,727	0,084
burglary of family household	0.74 ± 1.13	0.37 ± 0.66	1,458	0,145
murder of family member or friend	0.46 ± 1.19	0.52 ± 1.35	0,308	0,758
witness to murder	0.56 ± 1.43	0.27 ± 0.98	0,774	0,439
exposure to random shootings, terrorism, or riots	0.90 ± 1.66	0.40 ± 1.33	2,101	0,036
exposure to war or ethnic conflict	0.85 ± 1.92	0.20 ± 0.56	1.301	0.193
total score	8.32 ± 9.48	4.67 ± 6.01	2.301	0.021
Total score	33.60 ± 26.73	18.77 ± 16.49	3.022	0.003*

Z – Mann-Whitney U test; $p < 0.005$*; $p < 0.001$; SD – standard deviation.**

Discussion

Victimization is a complex concept with elements ranging from the more purely physical up to the emotional and sexual abuse. The majority of types of victimization occur in some form during the childhood.

There is the burgeoning literature on the association between the childhood victimization and the risk of suicidal behavior in the adolescence, but little is known about the association between children victimization and suicidal behaviors in the adulthood.

Differences can be explained by the fact that in adults, compared to young ones, early childhood experiences have lesser impact on suicidal behavior because of the greater flow of time and because unpleasant experiences from the childhood are deeply suppressed and replaced by other life experiences [17].

In our study, we examined the association between the childhood victimization and lifetime suicide attempts in a sample of patients hospitalized in the Day Hospital. The respondents of both groups were the patients on a psychotherapeutic treatment in the Day Hospital, who can recall long forgotten memories and suppressed the memories of traumatic events. So, in our survey, the children and adolescents were asked about suicidal attempts and whether they were abused in the childhood. The adult respondents, unlike the young respondents, were able to answer the questions about several forms of childhood abuse, including emotional and sexual and peer victimization.

The life-history data obtained from our patients who attempted suicide and received the psychotherapeutic treatment, pointed to the presence of victimization in their childhood. Our investigation showed that the experience of childhood abuse was a factor of suicidal risk in the adulthood. One of the reason the childhood abuse remains unrecognized has to do with the immature defensive systems that generate experiences of victimization during the childhood, where children are simultaneously the victims of violence of the same person (parents), who are also the people they rely on, which gives a false picture of real relationship, while psychotherapy provides the possibility of reminiscence of these events.

One of the common explanations for this relationship has to do with the immature defensive systems that generate experiences of victimization from the childhood. Injuries come from those who are trusted, which produces a tendency to believe that those who are trusted will be also those who will hurt him/her. This issue is particularly true in a small family system. When a child is abused by someone important to him/her, the child will have to give some explanation as to why this event happened. Given the fundamental nature of the parent-child relationship, the main purpose of the explanation will be to protect the perpetrator from liability. To this end, the child feels that the reason for the abuse has occurred. Many victims would say things like "Dad cannot make a mistake, but me." The result of these defenses is creation of guilt and shame. Attempts to commit suicide can be the act of acting out of guilt and shame [17, 18].

One possibility is that children do not have the ability to reduce their anxiety in ways that are available to adults, such as alcohol or cigarette, exercise in the gym, or excessive consumption of food. Frequent exposure to such victimization and physical maltreatment by adults, as well as bullying by peers, can lead the child to a critical attitude toward himself and use self-promotion as a means of self-indulgence [19–22].

Early exposure to victimization had results in a reduction of individual ability to cope with stressful situations, which increased their vulnerability and inability to deal with difficulties of life, when they chose maladaptive forms of behavior, including suicidal behavior [23–25].

Even after controlling for lifetime factors known to increase the suicide risk behavior, the adults who reported peer and sexual victimization in childhood were still more likely to attempt suicide later in life than other adults.

There are two explanations of dynamics of these relationships. The first reflects on the causal chain, where exposure to family and peer abuse increases the risk of problems in psychological and social functioning and the emergence of adolescent problems, or vice versa, the adolescent problems lead to an increased risk of suicidal behavior. Also, the childhood abuse results in a reduction in the individual ability to cope with stressful situations and adolescent problems, which can affect subsequent susceptibility to suicidal behavior [17, 18].

The persons who were victims later in adulthood can become violent, or (again) victims or both. Pain and anger over abuse can direct him\her to himself/herself or to someone else. With a good treatment and support they do not have to become either. When anger stays inside, a person can be self-destructive (self-inflicting, trying a suicide or other way to cause pain) or being injured by others. For those people, the self-destruction or suffering of pain inflicted by other people is more than necessary to make them feel powerful [18].

Our observations of childhood abuse show gender differences. In the childhood, male respondents were more often exposed to peer and sibling victimization, but women to sexual ones. The highest score on module of peer victimization had male respondents, while the highest values on module of sexual victimization had female respondents, which is in accordance with the literature data [23].

The statistical differences in the module of peer victimization were observed in bullying. The most current research findings shows that being involved in bullying in any way (as a person who bullies, a person who is bullied, or a person who both bullies and is bullied, i.e., bully-victim) is one of several important risk factors that appears to increase the risk of suicide among adults [6, 18].

Bullying and suicide-related behavior are both complex public health problems. There is evidence of a strong correlation between the childhood bullying with the risk of suicide later in life. Controlling for lifetime factors known to increase the risk of suicidal behaviour, adults who reported bullying in childhood were still more than twice as likely as other adults to attempt suicide later in life [6].

Bullying is associated with negative consequences. Circumstances that can affect a person’s vulnerability to either

or both of these behaviors exist at a variety of levels of individual, family, community and social influence. Being the victim of bullying involved them in the experience of suffering a defeat and humiliation that in turn could lead to hopelessness, entrapment, depression and suicidal behaviour [26]. Bullying co-occurred with several victimisation experiences including exposure to violence, sexual abuse, emotional distress, relationship problems, family conflict, and lack of connectedness to school as well as alcohol and drug use, physical disabilities, severe beatings and running away from home [27].

When explaining this phenomenon, we would see that the problem between the perpetrator and the victim is not a simple diadic relationship. Violence is a complex form of interpersonal aggression, which has many forms, serves to different functions and manifests itself in different ways. It is recognized as a phenomenon of the group, which occurs in a social context that promotes, supports or suppresses such behavior. Consequently, the behavior of the perpetrator is not only the result of the individual characteristics of the victim, but also under the influence of multiple connections with peers, family, teachers, neighbors and under the influence of the society as a whole. Cultural competence implies society's sensitivity to various factors affecting minority groups and immigrants such as stress migration, acculturation, history, poverty, language barriers, discrimination, taboos, values, beliefs and spirituality [28].

In peer and sibling victimization, the group chooses one person that is the most vulnerable, and he/she becomes a "scapegoat". It is the bearer of unconscious group guilt or malice, and its punishment releases members of the group of their sin through the release from aggravated aggression and the manifestation of that aggression towards those in whom such a manifestation will not have detrimental consequences for the perpetrators [29]. Thus, when a child hits or attacks, it becomes a victim of social violence. The attack follows various verbal statements, gossip, the spread of rumors, and finally the exclusion of a child from society [24].

In Sexual victimization module, there are high statistical differences in Statutory Rape & Sexual Misconduct. Results of our investigation are in accordance with the reseaches by other authors that show that in childhood, girls were more often exposed to sexual abuse than boys [30], and that sexual abuse increased the risk of multiple suicide attempts in adulthood [31]. Also, the women who had been sexually abused in the childhood were at a greater risk of committing suicide in adulthood [32].

And a few years after the sexual abuse, or the rape, women feel dirty, helpless, ruined, present with disgust toward themselves. Also, there is a shame, anger and feeling of guilt. Such a woman feels that she have done something different**,** and therefore she feels guilty. Or, she thinks something is wrong with her and the perpetrator was made to attack her because she deserved it. Self-indulgence prevents the process of healing of mental wounds after rape, which does not help recovery [13, 30–35].

Sexual abuse is not transmitted directly from the victim to the next generation, but rather relates to the dynamics of family functioning against sexual abuse [33, 36–39]. A girl or a woman who was raped, expected support from her closest ones. However, many of them were not always ready to provide this support, or some may not be willing to accept reality, and even women can blame, or abandon her and she then lose confidence in themselves and others. When a raped or a sexually abused woman does not have the ability to reduce her / emotions, she can attempt a suicide after her attempts to talk to others about her problems which were not successful overcame, and even more drastically, because she did not get the attention she needed. When anger stays inside, it can be self-destructive (self-inflicted, may try to commit a suicide or inflict pain to herself in other way), or be further harmed by others. For her self-destruction, or the suffering from pain inflicted on her by others, it is necessary to make her feel less guilty. When the anger is directed towards themselves, an attempted suicide can be a mechanism for acting out for guilt and shame. Self-discipline is a way of self-indulgence, but her pain would never heal [40–42].

Limitations of the study

Our investigation was done on a relatively small sample. In the Day Hospital of the Clinic of Psychiatry of the MMA in Belgrade approximately 110 patients were annually hospitalized which reflected to the number of patients involved in our investigation during the 3-year observation period.

This research could be continued and extended to a larger number of respondents, but in accordance with already mentioned, it requires many years of work on this problem.

It is necessary to conduct a national study, using the JVQ, on a large sample of adolescents in Serbia, to estimate the presence of various forms of victimization in childhood and early adolescents.

Conclusion

Significant suicide risk factors for commiting a suicide in adulthood are history of childhood peer and sibling and sexual victimization.

The ultimate goal of our prevention efforts is to reduce the suicide risk factors and increase the protective factors as much as possible.

Early recognition of the victims of children abuse could help them to adopt the effective strategies for overcoming maladaptive forms of behavior, which increases the ability to help a person successfully to overcome them and to prevent fatal consequences.

Learning the strategies of persons` experience, the opposite of some of the circumstances (family support rather than family conflict; strong school connectedness rather than lack of connectedness) are some of several “protective factors” that decrease suicide risk. In psychotherapy with patients following suicide attempt, the work on maturing defense mechanisms is very important.

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Received on August 26, 2017.
Revise on September 14, 2017.
Accepted on September 25, 2017.
Online First October, 2017.

ORIGINAL ARTICLE

UDC: 616.314-74
https://doi.org/10.2298/VSP161220146D

Shear bond strength to sound and caries-affected dentin of simplified "etch-and-rinse" and "self-etch" adhesives and the hybrid layer micromorphology

Mikromorfologija hibridnog sloja i otpornost na silu smicanja adhezivne veze formirane između pojednostavljenih potpuno nagrizajućih i samonagrizajućih adheziva sa zdravim i karijesno izmenjenim dentinom

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Abstract

Background/Aim. After removal of caries-infected dentin, a considerable area of the cavity floor comprising caries-affected dentin. Bonding to caries-affected dentin is characterized by lower bound strength and inferior hybrid layer guality compared to bonding to sound dentin. The purpose of study was to compare shear bond strength (SBS) of currently available adhesive systems to sound dentin (SD) and caries-affected dentin (CAD) and elucidate the hybrid layer micromorphology. **Methods.** Sixty extracted human molars with coronal carious lesions formed the experimental sample while additional sixty extracted intact human molars (impacted third molars) served as controls. Identification of a carious-affected dentin was carried out using visual identification (North Carolina Dentin Sclerosis Scale). Teeth from both the experimental and the control sample were allocated to one of the following three groups: Adper Single Bond Plus/Filtek Supreme XT (ASB/FS) (3M ESPE), AdheSE One/Tetric EvoCeram (AO/TEC) (Ivoclar Vivadent), and Prime&Bond NT/CeramX Mono (PB/CXM) (Dentsply). Bonding procedures utilized in this work were in line with the manufacturers' instructions. The SBS was measured using a universal testing apparatus. Hybrid layer micromorphology was observed under scanning electron microscope (SEM). The mean SBS values (MPa), and hybrid layer thickness (in μm) were statistically analyzed using the *t*-test, Mann-Whitney U-test, ANOVA, and Holm's test. **Results.** Mean SBS ± standard deviation were: ASB/FS to SD = 10.56 ± 3.49; ASB/FS to CAD = 10.06 ± 2.55; AO/TEC to SD = 7.01 ± 2.05; AO/TEC to CAD = 6.73 ± 1.66; PB/CXM to SD = 9.01 ± 2.47; PB/CXM to CAD = 7.83 ± 1.42. A statistically significant difference was found between the bonding strength of ASB/FS and AO/TEC to both SD and CAD, and between ASB/FS and PB/CXM to CAD. Hybrid layer thickness was statistically significantly greater for ASB/FS than for PB/CXM. For the ASB/FS system, a statistically significantly thicker hybrid layer was formed on CAD than on SD. No hybrid layer could be observed for AO/TEC. **Conclusion.** All tested compsite systems bond equally well on sound and caries-affected dentin. The etch-and-rinse adhesives achieved stronger bond strengths. The Adper single Bond Plus-Filtek Supreme XT system formed a statistically significantly thicker hybrid layer on both type 1 of dentin than the Primu 8 Bond NT-CeramX Mono system.

Key words:
dental caries; dentin; dentin bonding agents; adhesives; shear strength; materials testing.

Apstrakt

Uvod/Cilj. Uklanjanjem karijesno inficiranog dentina tokom preparacije kaviteta, značajne površine na njegovim zidovima ostaju pokrivene karijesno izmenjenim dentinom. Povezivanje sa karijesno izmenjenim dentinom karakteriše manja jačina veze i slabiji kvalitet gradnje hibridnog sloja. Cilj rada je poređenje otpornosti adhezivne veze na silu smicanja (SBS) aktuelnih adhezivnih sistema sa zdravim (SD) i karijesno izmenjenim dentinom (CAD), kao i analiza mikromorfologije hibridnog sloja. **Metode.** Šezdeset ekstrahovanih humanih molara sa karijesom okluzalne površine izabrani su kao eksperimentalna grupa, a šezdeset ekstrahovanih intaktnih humanih molara (impaktirani treći molari) izabrani su kao kontrolna grupa. Karijesno izmenjen dentin prepoznat je upotrebom vizuelne identifikacije (North Ca-

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rolina Dentin Sclerosis Scale). Zubi obe grupe podeljeni su u tri podgrupe zavisno od primenjenog kompozitnog sistema: Adper Single Bond Plus/Filtek Supreme XT (ASB/FS) (3M ESPE), AdheSE One/Tetric EvoCeram (AO/TEC) (IvoclarVivadent), Prime&Bond NT/CeramX Mono (PB/CXM) (Dentsply). Adhezivna procedura sprovedena je u skladu sa uputstvima proizvođača. SBS je merena na univerzalnoj kidalici. Mikromorfologija hibridnog sloja analizirana je upotrebom skening elektronskog mikroskopa (SEM). Otpornost adhezivne veze na silu smicanja u srednjim vrednostima (MPa) i debljina hibridnog sloja u μm statistički su analizirani pomoću *t*-testa, Mann-Whitney U-testa, ANOVA i Holmovog testa. **Rezultati.** Srednje vrednosti SBS ± standardna devijacija iznosile su: ASB/FS na SD = 10,56 ± 3,49; ASB/FS na CAD = 10,06 ± 2,55; AO/TEC na SD = 7,01 ± 2,05; AO/TEC na CAD = 6,73 ± 1,66; PB/CXM na SD = 9,01 ± 2,47; PB/CXM na CAD = 7,83 ± 1,42. Statistički značajna razlika uočena je između ASB/FS i AO/TEC na obe forme dentina: na zdravom i na karijesno izmenjenom dentinu, a između ASB/FS i PB/CXM samo na karijesno izmenjenom dentinu. Debljina hibridnog sloja je bila statistički značajno veća za ASB/FS nego za PB/CXM sistem. Kod sistema ASB/FS debljina hibridnog sloja je bila statistički značajno veća na CAD nego na SD. Nije uočeno postojanje hibridnog sloja kod AO/TEC sistema. **Zaključak.** Svi testirani kompozitni sistemi se jednako dobro vezuju sa zdravim i karijesno-izmenjenim dentinom. Tolhesivi sa potpunim nagrizanjem ostvaruju veće vrednosti jačine veze. Adper Single Bond Plus-Filtek Supreme XT system formira statistički značajno tanji hibridni sloj na oba tipa dentina u odnosu na Prime&Bond NT-CeramX Mono sistem.

Ključne reči:
zub, karijes; dentin; dentin, vezivna sredstva; adhesivi; smicanje; materijali, testiranje.

Introduction

After removal of caries-infected dentin, a considerable area of the cavity floor comprising caries-affected (CAD) dentin with partially demineralized collagen remains [1]. Since caries-affected dentin has different mechanical, physical, and chemical properties from those characterizing sound dentin (SD), achieving intimate adaptation of the resin composite and tooth tissue is significantly harder in the former case [2, 3].

For the purpose of implementing composite restoration adhesion to tooth tissue, it is important both for the primer and the resin to penetrate as deeply into demineralized dentin as possible, creating a structure known as a hybrid layer [4]. Hybrid layer quality depends on the adhesive chemical composition and the application technique used as well as the tooth region and presence of caries-affected dentin [5].

Contemporary adhesive systems may be classified as self-etch and etch-and-rinse (total-etch), according to the use of phosphoric acid as a surface etchant. The etch-and-rinse adhesives completely remove the dentin smear layer and smear plugs during acid conditioning. In the next step, resin penetrates into the demineralized zone and provides required adhesion [6]. However, this technique is very sensitive, potentially resulting in contamination due to inconsistencies in executing each step [7].

The self-etch adhesives contain acidic monomers that provide simultaneous conditioning and priming of tooth tissue [8]. They enhance adhesive interdiffusion through the smear layer. Therefore, this method is deemed user friendly (as it requires fewer steps) and less sensitive (as it does not involve wet-bonding) [9]. In terms of their pH, self-etch adhesives can be classified as: (a) 'ultra mild'(pH > 2.5), (b) 'mild'(pH ≈ 2), (c) 'intermediately strong'(1 < pH <2), and (d) 'strong'(pH ≤1) self-etch approach [8].

Adhesives can also be classified according to the number of steps required for their application, where by etch-and-rinse adhesives are divided into 3-step (separate application of acid, primer and adhesive resin) and 2-step (separate application of acid and mixture of primer and adhesive resin). Similarly, self-etching adhesives can be 2-step (separate application of self-etching primer and adhesive resin) and all-in-one (application of self-etching primer and adhesive resin in one solution).

Bonding to CAD is characterized by lower bond strength and inferior hybrid layer quality compared to bonding to SD, irrespective of the type of adhesive systemsemployed [10]. For the etch-and-rinse adhesives, discrepancies between the demineralization level and the extent of resin monomer infiltration have been reported. As conditioning with phosphoric acid cannot completely remove mineral deposits inside dentinal tubules, the resin infiltration depth can be compromised. The aforementioned issues contribute to lower bond strength [1]. The etch-and-rinse adhesives form a thicker hybrid layer on CAD relative to that on SD [11], with a greater prevalence of porous zones [3]. Owing to the complete removal of the smear layer and smear plugs by etch-and-rinse adhesives, a large number of resin tags is produced [6]. Self-etch adhesive systems have also demonstrated lower bond strengths to CAD compared to SD. During the application of self-etch adhesives, demineralization by acidic monomers and adhesive infiltration occur simultaneously. This approach results in fewer discrepancies in the level of demineralization and the resin monomer infiltration. The hybrid layer formed by self-etch adhesives on CAD is usually thicker than that formed on SD [1], with the less pronounced resin tag formation. Only strong self-etch adhesives form the typical resin tags in dentin [8].

The purpose of this study was to compare shear bond strength (SBS) of current adhesive systems to SD and CAD, as well as examine hybrid layer micromorphology. The null hypotheses were: 1) the tested composite systems bond equally well to sound and caries-affected dentin; 2) there is no difference between etch-and-rinse and self-etch adhesives in composite system in bonding to these respective substrates; 3) no important differences in the microstructure of the interfaces between the tested composite systems on respective substrates exist.

Methods

Tooth selection

Sixty caries-free and sixty molars with occlusal carious lesion were collected after obtaining the informed consent from the patients, as approved by the Committee of Ethics of the University of Novi Sad. Upon extraction, the teeth were cleaned with scalers and polished with pumice before being stored in 0.5% aqueous chloramine solution at 4 °C. After the seven-day storage period, the teeth were rinsed and transferred to distilled unionized water at 4 °C and were used within one month from extraction [12].

The caries-affected dentin identification procedure and macroshear bond testing

Using self-curing polyester resin, the roots of thirty molars with occlusal carious lesion (Group A) and thirty caries-free molars (Group B) were individually embedded in polyvinyl chloride (PVC) cylinders of 22 mm diameter and 20 mm height. The tooth crown of every specimen protruded from the cylinder. Enamel of all occlusal surfaces was then removed using a diamond bur (No:806 314110524014 NTI-Kahla, Gmbh, Germany) inserted in a high speed hand-piece under copious air-water spray. The exposed dentin surfaces of each specimen in the Group A were ground with silicon carbide abrasive papers (SiC 600-grit paper,3M) under running water using a custom made grinding cylinder (Figure 1) in order to obtain flat dentin surface perpendicular to the long axis of the tooth/PVC cylinder.

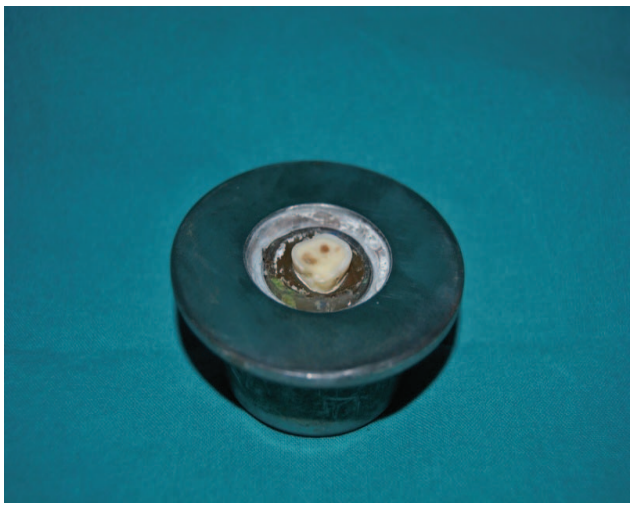

Fig.1 Custom made grinding cylinder.

To obtain caries-affected dentin, we ground the samples forming the Group B using the visual examination criteria set forth by the North Carolina Dentin Sclerosis Scale [13].

In each ground tooth, either SD or CAD was exposed, without revealing the pulp. Caries-affected dentin was identified according to the clinical (visual and tactile) examination guidelines, in accordance with the North Carolina Dentin Sclerosis Scale [13]. Opaque, light yellow, or whitish dentin was classified as sound dentin. Glassy dentin, dark yellow, or slightly brownish was classified as caries-affected dentin (Figure 2).

Fig. 2 – Caries-affected dentin.

The specimens from both groups were divided into three subgroups (n = 10), according to the adhesive/resin composite system used: Subgroup 1 – Adper Single Bond Plus/Filtek Supreme XT (3M-ESPE); Subgroup 2 – AdheSE One/Tetric EvoCeram (Ivoclar-Vivadent); Subgroup 3 – Prime&Bond NT/CeramX Mono (Dentsply).

Chemical composition and manufacturers' instructions for tested adhesives are shown in Table 1.

All implemented bonding procedures followed the manufacturers' instructions and the pertinent test protocol guidelines, based on the ISO/TS 11405 specification of the bonding area limitation [14]. The specimens were placed in a custom made tool−specimen bracket, comprising of a metal cube into which a cylindrical hole corresponding to the specimen dimensions (22 × 20 mm) was bored, and a transparent PVC cylinder of 4 × 4 mm dimensions, for placing the resin composite build-up (Figure 3). Assembling the afore mentioned two components in the described manner allowed forming the resin composite build-up perpendicular to the bonding dentin surfaces. These resin composite structures were layered gradually (in 2 mm increments), using the proprietary restorative resin composite of each adhesive. Each successive composite layer was light-cured using a LED curing device (SmarliteIQ2, Dentsply, Caulk, DE Milford, Serial No. B 21581).

Table 1

Chemical composition and manufacturers' instructions for tested adhesives

Adhesive	Chemical composition	Application
Adper Single Bond 2 (3M-ESPE, St Paul, MN,USA) LOT N172405	BisGMA, HEMA, dimethacrylates, ethanol, water, photoinitiator system, methacrylate functional copolymer of polyacrylic and polyitaconic acids	1. Apply Scotchbond Etchant (35%) to dentin. Wait 15 s and rinse for 10 s. Blot excess water using cotton pellet. 2. Immediately after blotting, apply 2–3 consecutive coats of adhesive for 15 s with gentle agitation using fully saturated applicator. Gently air thin for 5 s to evaporate solvent. Light-polymerize for 10 s.
AdheSE One (Ivoclar-Vivadent, Schaan, Liechtenstein) LOT M50900	Derivatives of bis-acrylamide, water, bis-methacrylamide dihydrogen phosphate, amino acid acrylamide, hydroxy alkyl methacrylamide, silicon dioxide, catalysts, stabilizers	Application and agitation for 30 s, followed by air dispersion until there is no water movement, and finally light-curing for 10 s.
Prime&Bond NT (Dentsply, Caulk, Milford, DE, USA) LOT 0905000886	Di- and trimethacrylate resins, PENTA (dipentaerythritolpenta acrylate monophosphate), nanofillers-amorphous silicon dioxide, photoinitiators, stabilizers, cetylaminehydrofluoride, acetone	Apply Conditioner 36 etch for 15 s. Rinse with water spray for 10s. applay soft blow of air, and ensuring that the surface remains moist. Saturate the surface with ample amounts of the adhesive, reapply if necessary. Leave the surface undisturbed for 20s. Air blow gently for 5s. Light cure for 10s.

Bis-GMA – BisphenolA-glycidyl methacrylate; HEMA – hydroxylethyl methacrylate.

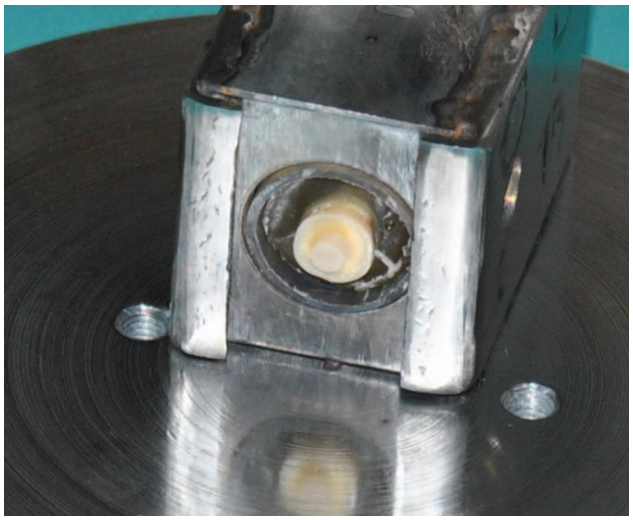

Fig. 3 – Specimen backet.

The prepared specimens were stored in distilled water at 37 °C for 24 h. Each specimen was used to determine the shear bond strength using the universal testing machine (Instron Testing Machine Model 1122, Instron, Norwood, Massachusetts, US). Prior to testing, the specimens were secured in a specimen bracket fixed to the universal testing apparatus. A straight-edge guillotine was positioned as close as possible to the bonding interface and was aligned with the loading axis of the testing construction. A crosshead speed of 1 mm per minute was used. The shear stress was recorded in Newton (N), and the bond strength was calculated using the following equation: P (MPa) = F [N/S (mm^2)] [15, 16].

The shear bond strengths were analyzed using the *t*-test, Mann-Whitney U-test, two-way ANOVA, and Holm's test, available in the Primer of Biostatistics Statistical Software Program. In the two-way ANOVA analysis, the adhesive/composite group and dentin substrate were the tested factors and the level of significance was set to $p < 0.05$.

Scanning electron microscopy (SEM) evaluation

The scanning electron microscopy evaluation was conducted on 60 extracted human molars, divided into two groups according to the previously described criteria. Bonding substrates were prepared using the same procedure as that adopted shear bond strength testing. Once again, bonding procedures were in line with the manufacturers' instructions (Table 1), and composite structures were constructed in bulk, in one 2 mm increments, applying the proprietary restorative resin composite of each adhesive. The teeth were then stored in distilled water at 37 °C for 24 h. The bonded teeth with the composite build-ups were sectioned parallel to the bonded surface to expose the dentin-adhesive interface. The specimens were polished under running water using silicon carbide (SiC) grinding papers of increasingly finer grit (600, 1000 and 1200). For each polished specimen, the CAD area was marked with a #11 scalpel under magnifying glass. The specimens were treated with 32% silica-free phosphoric acid gel (Uni-Etch, Silica gel free, Bisco, Schaumburg, IL, LOT 0800012148) for 60 s, followed by immersion in 2% sodium hypochlorite for 60 s to expose dentin-adhesive interface [17]. After rinsing with distilled water, the specimens were prepared under the Environmental scaning electron microscope (E-SEM) protocol, under the low vacuum and in wet conditions [18]. The specimens were examined by the SEM (JEOL, JSM-6460 Low Vacuum, Tokyo, Japan) at 1000 × magnification [17].

Table 2

The mean shear bond strength (SBS) ± standard deviation (sd) values, presence of statistical significance between the groups

Adhesive	Mean ± sd (min, max) in MPa CAD	Mean ± sd (min, max) in MPa SD	Significance CAD vs. SD
1. ASB-FS	10.06 ± 2.55 (5.09, 12.89)	10.56 ± 3.50 (6.37, 16.51)	non-significant
Significance 1 vs. 2	$p < 0.01$	$p < 0.02$	
2. AO-TEC	6.73 ± 1.66 (4.93, 10.34)	7.00 ± 2.05 (4.61, 11.3)	non-significant
Significance 2 vs. 3	non-significant	non-significant	
3. PB-CXM	7.83 ± 1.42 (5.25, 10.19)	9.0 ± 2.47 (4.46, 12.41)	non-significant
Significance 1 vs. 3	$p < 0.03$	non-significant	

SD – sound dentin; CAD – caries affected dentin; ASB-FS – Adper Single Bond Plus-Filtek Supreme XT.

Results

Shear bond strength (SBS) testing

Descriptive statistics pertaining to the shear bond strengths and the statistical significance of between-group differences are presented in Table 2.

The results yielded by our investigation showed that the adhesive system was a significant factor in determining shear bond strength. Greater bond strengths were achieved using the etch-and-rinse adhesives. The lowest bond strengths were noted for the AdheSE One-Tetric EvoCeram combination on CAD. In addition, the obtained value was statistically significantly lower than that measured for the Adper Single Bond Plus-Filtek Supreme XT combination applied to both CAD ($p < 0.01$) and SD ($p < 0.02$) specimens. Prime&Bond NT-CeramX Mono exhibited statistically significantly lower bond strength on CAD than the Adper Single Bond Plus-Filtek Supreme XT ($p < 0.03$). On the other hand, the bond strengths of Prime&Bond NT CeramX Mono and AdheSE One Tetric EvoCeram were not statistically significantly different. Finally, none of the examined adhesives exhibited statistically significantly different shear bond strengths to sound dentin relative to caries-affected dentin ($p > 0.05$).

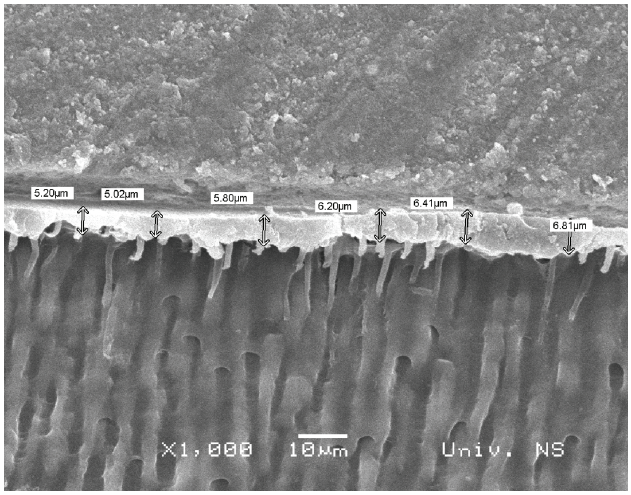

Fig. 4 – Scaninning electron micrograph at adhesive bond between Adpersingle Bond2/Filtek supremeXT to caries affected dentin (1000x magnification).

SEM analysis

The hybrid layer thickness was measured using the SEM device (NIH Image Analyser) proprietary software (Figures 4–7).

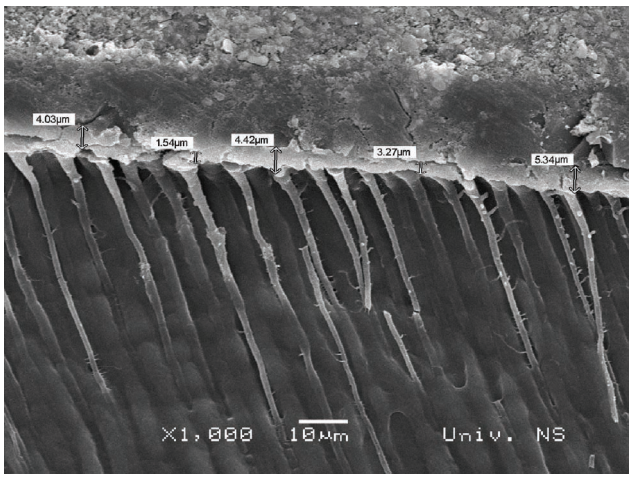

Fig. 5 – Scanning electron micrograph of adhesive bond bectween AdperSingle Bond2/Filtec supremeXT to sound dentin (SD) (1000x magnification).

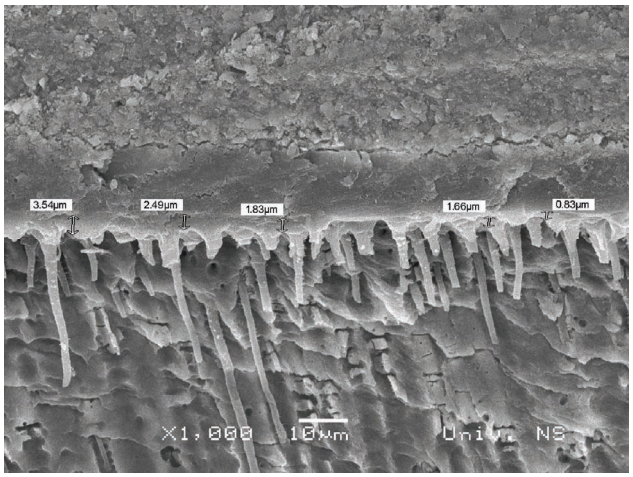

Fig. 6 – Scanning electron microgoph (SEMg) of adhesive bond between Prime&BoundNT/CeramX Mono to caries affected dentin (CAD) (1000x magnification).

Table 3

Basic statistical parameters characterizing hybrid layer thickness (HLT) and presence of statistical significance

Adhesive	Mean HLT ± sd (min, max) in μm CAD	Mean HLT ± sd (min, max) in μm SD	Significance CAD vs. SD
1. ASB-FS	5.32 ± 1.54 (2.89, 8.16)	3.39 ± 0.51 (2.23, 3.84)	$p < 0.01$
Significance 1 vs. 2	$p < 0.05$	$p < 0.05$	
2. PB-CXM	3.21 ± 2.68 (1.67, 4.54)	2.68 ± 0.91 (1.61, 4.44)	non-significant

sd – standard deviation; CAD – caries affected dentin; SD – sound dentin; ASB-FS – Adper Single Bound Plus-Filtek Supreme XT; AD-TEC – AdhesEOne-Tetric Evo Cerani; PB-CXM – Prime&Boudnt-CeramX Mono.

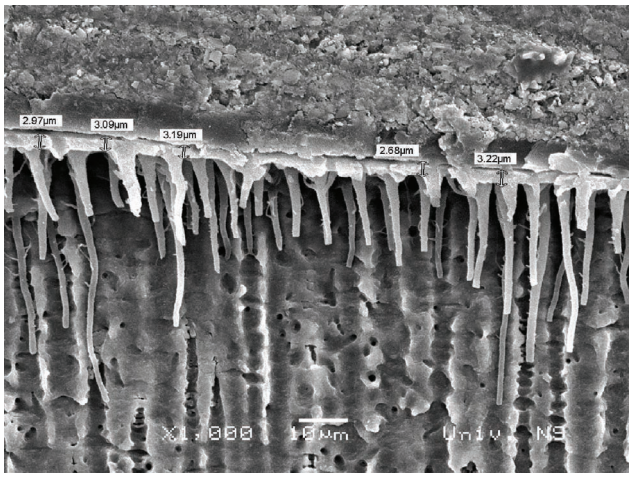

Fig. 7 – Scanning electron micrograph of adhesive bond between Prime&BondNT/CeranXMono to sound dentin (SD) (1000x magnification).

SEM images revealed that AdheSE One Tetric EvoCeram did not form the hybrid layer on the examined specimens.

The main statistical parameters pertaining to the hybrid layer thickness are presented in Table 3, along with the statistical significance of between-group differences. As can be seen from the tabulated findings, the greater hybrid layer thickness was formed on the CAD specimens.

The Adper Single Bond Plus-Filtek Supreme XT system produced a statistically significantly thicker hybrid layer on both types of dentin than the Prime&Bond NT – CeramX Mono system. On the SD specimens, the hybrid layers were thinner and the difference was statistically significant for the Adper Single Bond Plus-Filtek Supreme XT system only. No hybrid layer could be observed on the specimens with AdheSE One-Tetric EvoCeram.

Discussion

Adhesion to dentin is an important step in placing composite restoration. Organic nature of dentin and its higher humidity relative to enamel makes bonding to this hard tissue very difficult[2]. Compared to SD, bonding to CAD is characterized by the lower bond strength and inferior quality of the hybrid layer [10]. Owing to these discrepancies, the bonding efficacy of different adhesive systems with SD and CAD was extensively studied [18].

Development of new adhesive systems requires valid laboratory methods for testing material properties, such as the shear bond strength test technique [19]. To standardize the test protocol, ISO/TS 11405 specification was established in 2003. The device recommended by this protocol is referred to as a "guillotine" [14]. In our study, the adhesives that were compared in terms of their bond strength included the corresponding composite provided by the same company. Consequently, conclusions can only be drawn at the level of the adhesive/composite combination [20]. According to Salz and Bock [21], the "comparative bond strength tests are possible only at the level of identical adhesive/composite combinations, and certainly not at the level of the adhesive alone". This assertion justifies the approach adopted in our investigation. Indeed, the goal of testing adhesives incorporating composites produced the same company is to inform their application in everyday practice.

In our study, the comparison of the shear bond strengths of each adhesive to different substrates failed to reveal statistically significant differences. Consequently, the first null hypothesis was accepted. The shear bond strengths to SD and CAD were comparable, as the noted differences were not statistically significantly different. These results are in accordance with those reported by other authors [13, 20]. For example, Pereira et al. [22] posited that the absence of statistically significant differences between the bond strengths of Adper Single Bond Plus adhesive with SD and with CAD can be attributed to the large standard deviations, operator variability and/or technique sensitivity of this adhesive. Sonoda et al. [23] also reported the absence of statistically significant differences in the bond strength value of Prime&Bond NT to SD and CAD. These authors suggested that the caries retained after excavation, rather than the adhesive bond interface itself and this is potentially the weakest part of the adhesive bond. The low bond strength values were reported for AdheSE One to SD and CAD in other investigations, in which statistically significant differences between the two could not be established [17, 24]. AdheSE One has a pH of 1.5 and is classified as an "intermediately strong" self-etch approach (pH in the1−2 range) [8]. The functional monomer type in the adhesive composition plays a crucial role in the self-etch adhesive performance. In AdheSE, bis-methacrylamide dihydrogen phosphate serves as a functional monomer. This molecule is characterized by a short spacer chain of phosphate functional monomer, which induces formation of unstable monomer calcium salts. Consequently, chemical interactions are less pronounced, resulting in a lower dentin bond strength [25]. AdheSe is classified as a 2-hydroxylethyl methoacrylate (HEMA) free adhesive. Presence of HEMA in the self-etch

adhesive composition increases the bond strength to dentin, as it provides good dentin wetting and hinders a phase separation between the hydrophobic components and dentin [25]. The low bond strength values of these adhesives can thus be attributed to the nature of their polymerization within dentin. Namely, a photoinitiator used in AdheSE One may contain acylphosphine oxides that do not react with many of the newer light emitting diode (LED) light curing units [23, 26].

A comparison of the SBS of the tested composite systems revealed some statistically significant differences (Table 2). Consequently, the second null hypothesis was rejected. Specifically, the bond strength of composite system with etch & rinse adhesive (Adper Single Bond Plus+Filtek Supreme XT) was statistically significantly higher than that measured for the composite system with self-etch adhesive (AdheSE One+Tetric EvoCeram). These results correspond to the findings yielded in an extensive study in which more than 16,000 SBS tests were examined [27]. However, as no statistically significant differences were noted between the bonding strength of the composite system with etch & rinse adhesive (Prime&Bond NT+ CeramX Mono) and that of the composite system with self-etch adhesive (AdheSE One+Tetric EvoCeram), our findings are not in line with those reported by Degrange and Lapostolle [27]. No differences between the shear bond strength of Prime&Bond NT and self-etch adhesives were found in the study conducted by Li et al. [28]. Prime&Bond NT is two-step acetone based etch & rinse adhesive characterized by high technique sensitivity. Acetone is unable to re-expand shrunken demineralized collagen [29]. High technique sensitivity and the chosen bond testing method (macro shear) may result in the low bond strength values [30, 31].

SEM is typically used when investigating the bonding mechanisms [18]. However, the ultrastructural data pertaining to adhesive interface cannot be directly interpreted in terms of bond strength to the tooth tissues. Micromorphological findings should always be carefully explained, as microscopic observations do not always correspond to the clinical findings [17].

Adhesion to dentin is a critical step in adhesive procedure. When using etch & rinse adhesives, the process commences with acid conditioning of dentin using phosphoric acid. This step results in a complete removal of the smear layer and smear plugs, leading to dentinal surface demineralization and exposure of the collagen fibrils [32]. The second step comprises of a resin monomer penetration into this demineralized dentinal surface. As a result, a resin-matrix reinforced by collagen fibrils, called hybrid layer, is formed [6]. Penetration of the resin monomer into opened dentinal tubules leads to the resin tag formation. The hybrid layers formed with CAD are thicker than those of SD [1–3, 5, 27, 33]. As CAD is partially demineralized, it is more susceptible to acid-etching. This leads to the formation of a deeper demineralized zone. For both CAD and SD, discrepancies the between demineralization depths and the resin monomer penetration extent are common [1]. The presence of highly acid resistant mineral deposits in dentinal tubules would interfere with the resin monomer infiltration as well as the resin tag formation. Thus, hybrid layer formed with CAD is thicker and the resin tags are less numerous, while the lateral branches are less pronounced and shorter.

Self-etch adhesives form a thicker hybrid layer with CAD compared to SD, but are thinner than those obtained when etch & rinse adhesives are utilized [3, 33]. The typical resin tags in dentin are produced by strong self-etching adhesives ($pH \leq 1$) only, whereas they are rarely formed when mild and ultra-mild self-etching adhesives are employed [8]. Since the AdheSE One is classified as an "intermediately strong" self-etch approach, the typical resin tags are less numerous and are characterized by the poor lateral branches (Figure 8).

Due to their pH, self-etching adhesives cannot dissolve acid-resistant mineral deposits in dentinal tubules of CAD. However, the adhesive monomer penetration into CAD is hindered by a deeper mineralized zone, rather than adhesive pH [1]. With the exception of those based on strong self-etching adhesives, these systems cannot dissolve the smear layer and smear plugs, and they remain as a part of hybridized complex.

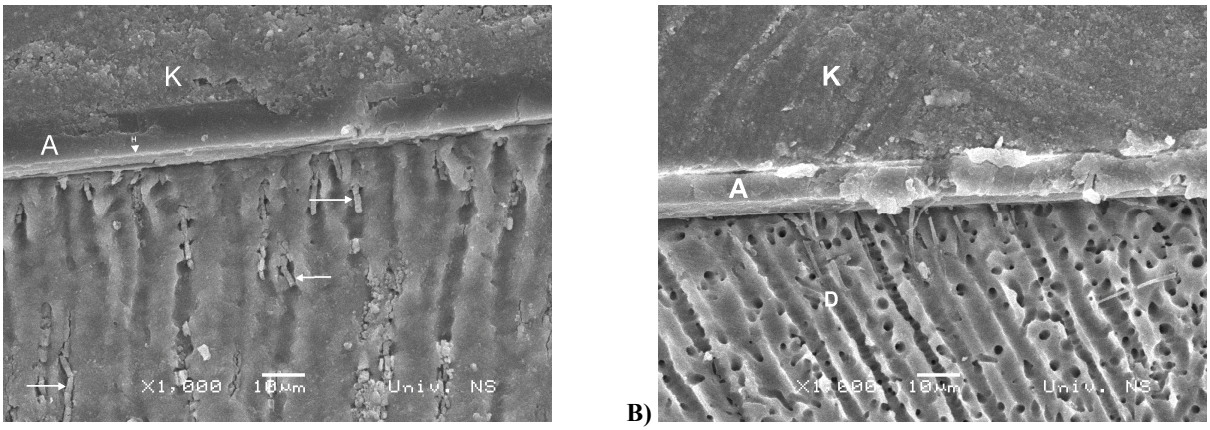

Fig. 8 – Scanning elecron micrograph (SEMg) of dentin specimens bonded with AdheSE One (1000x magnification). A) Caries affected dentin (CAD): barely observable hybrid layer, limited number of resin tags (arrows); B) Sound dentin (SD) – absence of hybrid layer, negligible presence of resin tags, many open dentin tubules without resin infiltration.
K – composite; A – adhesive layer; H – hybrid layer; D – dentin.

As the smear layer of CAD is enriched with disorganized collagen and mineral deposits, it may obstruct the resin monomer infiltration [34]. Thus, as we have found important differences in the microstructure of the interfaces between the tested composite systems on respective substrates, the third null hypothesis was rejected.

Conclusion

Noting the limitations of our study, the following conclusions can be drawn: 1) all tested composite systems bond equally well on sound and caries-affected dentin; 2) the etch-and-rinse adhesives achieved the stronger bond strengths; the Adper Single Bond Plus-Filtek Supreme XT system formed a statistically significantly thicker hybrid layer on both types of dentin than the Prime&Bond NT-CeramX Mono system.

Acknowledgement

This research is supported by Serbian Ministry of Education, Science and Technological Development projects 174005 and III44003.

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Received on December 20, 2016.
Revised on September 13, 2017.
Accepted on September 28, 2017.
Online First October, 2018.

ORIGINAL ARTICLE

UDC: 616.233-002-08
https://doi.org/10.2298/VSP170731153P

Inappropriate prescribing of antibiotics to the patients with acute bronchitis

Neadekvatno propisivanje antibiotika bolesnicima sa akutnim bronhitisom

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Abstract

Background/Aim. Inappropriate prescribing of antibiotics to the patients with acute bronchitis is frequent event in clinical practice with potentially serious consequences, although majority of treatment guidelines do not recommend it. The aim of this study was to reveal risk factors associated with inappropriate prescribing of antibiotics to the patients with acute bronchitis in primary healthcare. **Methods.** This case/control study included the adult patients with acute bronchitis during the initial encounter with a general practitioner. Prescription of an antibiotic was an event that defined the case, and patients without prescribed antibiotic served as controls. **Results.** Antibiotics (mostly macrolides and beta-lactams) were prescribed to the majority of patients with diagnosis of acute bronchitis (78.5%). A significant association was found between antibiotic prescription rates and patient age, whether an attending physician is a specialist or not and the average number of patients a physician sees per day [$OR_{adjusted}$was 1.029 (1.007–1.052), 0.347 (0.147–0.818) and 0.957 (0.923–0.992), respectively]. **Conclusion.** When there is primary care encounter with patients suffering from acute bronchitis, older patients are more likely to receive inappropriate antibiotic prescription, especially if their physician is without specialist training and has less patient encounters in his/her office daily.

Key words:
bronchitis; anti-bacterial agents; risk factors; comorbidity; medication errors; serbia.

Apstrakt

Uvod/Cilj. Neadekvatno propisivanje antibiotika bolesnicima sa akutnim bronhitisom česta je pojava u kliničkoj praksi, sa potencijalno ozbiljnim posledicama, iako većina vodiča za lečenje akutnog bronhitisa to ne preporučuje. Cilj naše studije bio je da se doprinese postojećim saznanjima o neodgovarajućem propisivanju antibiotika. **Metode.** Ova studija tipa slučaj/kontrola uključivala je odrasle bolesnike sa akutnim bronhitisom tokom prvog susreta sa lekarom opšte prakse. Propisivanje antibiotika je definisalo slučajeve, a bolesnici bez propisanog antibiotika služili su kao kontrola. **Rezultati.** Antibiotici (uglavnom makrolidi i beta-laktami) bili su propisani većini bolesnika sa dijagnozom akutnog bronhitisa (78,5%). Uočena je značajna povezanost između propisivanja antibiotika i starosti bolesnika, između propisivanja antibiotika i toga da li je lekar specijalista ili ne, kao i između propisivanja antibiotika i prosečnog broj bolesnika koje lekar pregleda tokom dana. Prilagođeni odnos šansi bio je [1,029 (1,007–1,052), 0,347 (0,147–0,818) i 0.957 (0.923–0.992), respektivno]. **Zaključak.** Prilikom prvog susreta bolesnika sa dijagnozom akutnog bronhitisa veću šansu da im antibiotik bude neadekvatno propisan imaju stariji bolesnici, posebno ako lekar nije specijalista i ako ima manji prosečan broj bolesnika u svojoj ordinaciji tokom dana.

Ključne reči:
bronhitis; antibiotici; faktori rizika; komorbiditet; lečenje, greške; srbija.

Introduction

Inappropriate antibiotic prescribing has been recognized as an important public health problem worldwide [1], because it contributes to development of antimicrobial resistance [2] According to the World Health Organization (WHO), inappropriate drug prescribing is denoted by unnecessary prescribing (overprescribing), omission, wrong selection of antibiotic, wrong dosage, incorrect duration of treatment, unnecessary expenses and unnecessary risk [3]. Any prescription event should be in accordance with the available evidence-based guidelines. A recent large cross-sectional survey con-

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ducted in the United States found that 30% of outpatients oral antibiotic prescriptions, regardless of indications, could have been inappropriate, and for acute respiratory infections up to 50% of prescribed antibiotics could have been unnecessary [4]. Among the acute respiratory tract infections, the highest percentage of inappropriate antibiotic prescribing occurs in the adult patients with acute bronchitis[4]; one of the studies found that almost 80% of these patients had antibiotics prescribed, but inappropriateness rate was 100% [5].

Acute bronchitis is a self-limiting inflammation of the large airways – bronchi, accompanied by a cough (productive or not) which can last up to 6 weeks, with absence of tachycardia, tachypnea, fever and abnormal findings on the chest examination [6]. In most cases, acute bronchitis is a viral infection. The most recent recommendations by the American College of Physicians state that antibiotic therapy should not be initiated in the patients with acute bronchitis, unless pneumonia is suspected [6]. Other available evidence-based guidelines also do not recommend prescription of antibiotics for acute bronchitis [7,8]. A trend of increased adverse events rate in the acute bronchitis patients treated with antibiotics was found in a systematic review of 17 randomized clinical trials [8].

Recent studies have shown that many factors may have an influence on inappropriate prescription of antibiotics [9]. These factors include a patient's history (comorbidities, age), clinical experience of physicians and a type of specialization [10]. Socio-economic factors, closely related to aspects such as the healthcare funding and reimbursement, number of generic drugs on the market and availability of diagnostic tests were also recognized as important [2]. Some of the studies also showed relevance of patient's demand and satisfaction, doctor's unwillingness to accept uncertainty and risks and fear of complications [9,11]. Findings from a cross-national study which included the data on prescription of antibiotics in primary care in 26 European countries demonstrated a significant correlation between antibiotic resistance and outpatient antibiotic use [12]. Rational use of antibiotics is necessary in order to preserve their effectiveness in circumstances of growing antimicrobial resistance [1].

Despite the relevance of this problem, many issues in regard to the factors influencing inappropriate prescribing of antibiotics to the patients with acute bronchitis remain unresolved. The aim of this study was to reveal risk factors associated with inappropriate prescribing of antibiotics to the patients with acute bronchitis in primary healthcare.

Methods

The study included the adult patients with acute bronchitis (the code J20 according to the International Classification of Diseases, 10th version: ICD-10) during the initial encounter with a general practitioner at the Primary Health Care Centers at three cities in the Republic of Serbia (Kragujevac, Novi Pazar and Šabac) and one city in Republic of Montenegro (Bijelo Polje). The patients were treated at the Primary Health Care Centers during the year 2016.

This study was designed as an analytical, clinical observational, case/control study. There were two groups in the study: the group of cases, who were prescribed an antibiotic at the initial encounter, and the group of controls, who were treated without an antibiotic.The data were collected from the patient files and the personal files of physicians employed at the Primary Health Care Centers. In each of the four cities the offices of Primary Health Centers already designated with number 1 or as “the first” were included in the study. The patients were enrolled consecutively, as their files were found in a patient registry of the health facilities where the study took place, starting from the index date of the study: January the 1st, 2016. Based on expected power of the study of at least 0.8, and on probability of type I error of maximum 0.05, the sample size was set on 200 patients. Further collection of data on the study sites was terminated after the target of 200 patients was reached. The last patient who entered the study was treated due to acute bronchitis on November the 17th, 2016. The study was approved by the local Ethics Committee (decision No 01-569/2, 26.01.2017.).

Inclusion criteria for the study patients were: both genders 18–70 years of age, diagnosis of acute bronchitis (ICD-10 code J20) established by a general practitioner and good general condition. The exclusion criteria were as the following: tachycardia (heart rate above 100 beats per minute), tachypnea (respiration rate above 24 per minute), fever above 38°C, abnormal respiratory sounds, age below 18 or above 70 years, chronic obstructive pulmonary disease – COPD, the patients undergoing chemotherapy or taking immunosuppressant, patients with asplenia, patients with hematological malignancy and pregnant or lactating women.

The following data were extracted from the patient or personal files: educational level of prescribers (general practitioner or specialist of general medicine); working experience in years; duration of employment at the study site; average number of patient encounters per physician per day; patients’ gender; age; employment status of patients; the number of times the patients visited a physician in 2016; the Charlson Comorbidity Index; a patient being hypertensive or not; concomitant therapy: ACE inhibitors, diuretics, beta blockers, statins, other cardiovascular drugs, antidepressants, antipsychotics, sedatives, anticonvulsants or anticoagulants.

The study data were analyzed by the descriptive statistics and presented in tables. Mean was used as a measure of central tendency and standard deviation as a measure of dispersion for continuous variables. The values of categorical variables were presented as rates or percentages. After checking the normality of the data distribution for the continuous variables (Kolmogorov-Smirnov test), an appropriate parametric or nonparametric tests were applied (Student's *t*-test for independent samples or Mann-Whitney *U* test). Significance of differences in the rates of categorical variables’ values were tested by the χ^2 test, or in case of low prevalence of particular categories by the Fisher’s test. Null hypothesis was considered to be true if probability of difference was less than 0.05. An influence of potential risk factors on inappropriate prescribing of antibiotics was evaluated by the univariate and multivariate binary logistic regression analysis. The results were shown as crude and adjusted odds ratios (ORs) with corresponding 95% confidence intervals (CI). All

calculations were performed by the statistical program for social sciences (SPSS version 18).

Results

The study included 200 patients with acute bronchitis. There were 157 patients to whom antibiotics were prescribed (cases). The most common prescribed class of antibiotics were macrolides [66 (42.1%) patients], followed by beta lactam antibiotics [52 (33.1%) patients] and beta lactam antibiotics with beta-lactamase inhibitor [15 (9.5%) patients]. Baseline characteristics of cases and controls are shown in Table 1. Significant differences between cases and controls were observed in the following features: the patients who got an antibiotic prescription were older ($p = 0.012$), had higher Charlson Comorbidity Index ($p = 0.044$) and were treated by an attending physician who did not have a specialization ($p = 0.032$).

Table 1

Baseline characteristics of cases and controls

Variable	Cases (n = 157)	Controls (n = 43)	Test value and significance of null hypothesis
Age of the patient (years), mean ± SD	49.4 ± 16.7	42.1 ± 16.6	$U = 2528.5$ $p = 0.012$*
Gender of the patient, n (%)			
female	90 (57.3)	27 (62.8)	$\chi^2 = 0.221$
male	67 (42.7)	16 (37.2)	$p = 0.638$
Employment status of the patient, n (%)			
unemployed	30 (20.3)	14 (32.6)	$\chi^2 = 5.385$
employed	61 (41.2)	20 (46.5)	$p = 0.068$
retired	57 (38.5)	9 (20.9)	
Number of the patient's visits to the doctor in the previous 12 months, mean ± SD	8.3 ± 5.7	7.3 ± 6.1	$U = 2933.5$ $p = 0.187$
Hypertension, n (%)	74 (47.1)	14 (32.6)	$\chi^2 = 2.349$ $p = 0.125$
Charlson Comorbidity Index	1.2 ± 1.5	0.9 ± 1.8	U = 2741.5 $p = 0.044$*
Chronic drug therapy†, n (%)	98 (62.4)	22 (51.2)	$\chi^2 = 1.344$ $p = 0.246$
The data about prescribers			
Age of the attending physician (years), mean ± SD	49.8 ± 6.9	49.6 ± 6.4	$U = 3120.0$ $p = 0.445$
Gender of the attending physician, n (%)			
female	152 (96.8)	42 (97.7)	$\chi^2 = 0.085$
male	5 (3.2)	1 (2.3)	$p = 0.770$
Physician is a specialist, n (%)	71 (45.2)	28 (65.1)	$\chi^2 = 4.578$ $p = 0.032$*
Working experience of the physician (years), mean ± SD	21.4 ± 8.8	21.9 ± 8.0	$U = 3369.0$ $p = 0.985$
Number of the patients who chose the attending physician, mean ± SD	1,623.0 ± 302.5	1,601.8 ± 245.0	$U = 3374.0$ $p = 0.996$
Average number of the patients the physician sees *per* day, mean ± SD	32.9 ± 10.3	35.7 ± 11.5	$U = 2781.5$ $p = 0.074$
Distribution of cases and controls according to the study site, n (%)			
Kragujevac	111 (71)	29 (68)	
Novi Pazar	17 (11)	3 (7)	$\chi^2 = 0.367$ $p = 0.947$
Šabac	13 (8)	7 (16)	
BijeloPolje	16 (10)	4 (9)	
Total	157 (100)	43 (100)	

SD – standard deviation; * – statisticaly significant; † – any of the following: statin, anticoagulant, ACE inhibitor, diuretic, beta blocker, or other cardiovascular drug, antidepressant, antipsychotic, sedative, antiepileptic drug.

Table 2

Crude and adjusted odds ratios (OR) of the risk factors for antibiotic prescribing

Risk factors	Crude OR (95% CI)	*p*	Adjusted# OR (95% CI)	*p*
Age of the patient	1.026 (1.005–1.047)	0.013*	1.029 (1.007–1.052)	0.010*
Attending physician is a specialist	0.442 (0.219–0.892)	0.023*	0.347 (0.147–0.818)	0.016*
Average number of the patients the doctor sees per day	0.976 (0.946–1.008)	0.139	0.957 (0.923–0.992)	0.015*

 # – adjusted for age of the physician, attending physician is a specialist, working experience of the physician, average number of the patients a physician sees per day, number of the patients who chose the attending physician, age of the patient; CI – confidence interval; * – statistically significant.

Table 3

The interactions between significant risk factors for antibiotic prescribing

Risk factors	Crude OR (95% CI)	*p*	Adjusted# OR (95% CI)	*p*
Age of the patient and attending physician is a specialist	0.991 (0.979–1.003)	0.157	0.963 (0.920–1.009)	0.117
Age of the patient and average number of the patients a physician sees per day	1.000 (1.000 – 1.001)	0.297	1.004 (1.001–1.006)	0.004*
Attending physician is a specialist and average number of the patients a physician sees per day	0.975 (0.958–0.993)	0.007*	1.035 (0.952–1.127)	0.419

 # – adjusted for age of the physician, attending physician is a specialist, working experience of the physician, average number of the patients a physician sees per day, number of the patients who chose the attending physician, age of the patient; OR – odds ratio; CI – confidence interval; * – statistically significant.

The results of both univariate and multivariate stepwise backward conditional binary logistic regression from the last step with satisfactory goodness of fit (Cox & Snell R square 0.097, Nagelkerke R^2 0.149, Hosmer-Lemeshow χ^2 14.356, df = 8, $p = 0.073$) with adjustment for potential confounders are shown in the Table 2. The variables entered the multivariate analysis were: age of the attending physician, gender of the physician, a physician is a specialist, working experience of the physician, an average number of patients the physician sees per day, a number of patients who chose the attending physician, age of the patient, gender of the patient, a number of patient's visits to the physician in the previous 12 months and the Charlson Comorbidity Index. A statistically significant association with the inappropriate antibiotic prescribing was found for the following variables: age of the patient, physician is a specialist and an average number of the patients the physician sees per day. The older patients were more likely to get an antibiotic prescription, whereas the patients who were treated by a physician who was a specialist and who was seeing a larger number of the patients per day were less likely to get an antibiotic prescription.

The interactions between significant risk factors for getting an antibiotic prescription were investigated (Table 3). A significant interaction was observed between the age of the patient and the average number of patients the physician was seeing per day after the adjustment for potential confounders. The odds ratio for interaction between being treated by a physician with specialization and the average number of patients the physician was seeing per day after adjustment dropped down and its confidence interval included 1, was no longer statistically significant ($p > 0.05$).

Discussion

The results of our study showed that physicians prescribe antibiotics to majority of patients with diagnosis of acute bronchitis (78.5 %). Macrolides were prescribed the most frequently, while beta-lactams were in the second place. A significant association was found between the antibiotic prescription rates and patient age, whether attending physician is specialist or not and average number of the patients a physician sees per day. Antibiotics were prescribed to the older patients more frequently. On the other hand, antibiotics were less often prescribed by the physicians who are specialists and who had a larger number of the patients per day.

Majority of guidelines for appropriate use of antibiotics suggest that antibiotics should not be prescribed to the patients with acute bronchitis at first encounter [6, 8]. Despite these recommendations, physicians continue to prescribe antibiotics to many patients with acute bronchitis [13]. The prescribing rate in our study is not discordant with other reports, where over 65% of patients with acute bronchitis received antibiotics [10, 14, 15]. Macrolides are the most commonly pre-

scribed antibiotics to the patients with acute bronchitis, being either on the first [16] or second place [10, 17, 18] according to absolute volume of prescriptions, competing only with beta-lactams. This is not surprising, considering that these antibiotic groups are active against the majority of bacteria causing outpatient respiratory infections [6]. The probable reason for such behavior of prescribers could be fear of missing bacterial infection if abstaining from antibiotics and of consequent deterioration of patient condition which may be ascribed to the prescriber as neglect or professional mistake. The prescribers than could snatch at antibiotics to ensure treatment efficacy, counting on relatively low rate of adverse effects in this group of drugs.

The association between antibiotic prescribing and the age of patients with acute bronchitis (older patients are more likely to get an antibiotic prescription) found in our study was also reported by others [16, 19–21]. Kroening-Roche et al. [21] showed that the patients being 50 and older had 1.7 times higher chance to receive antibiotic for acute bronchitis than younger patients. A reason for more frequent prescribing of antibiotics to elderly patients with acute bronchitis is a fear of misdiagnosis or complications like pneumonia, which has a high mortality rate in the elderly [13]. The elderly patients are also more expectant than the younger ones, which builds up the pressure on prescribers who may be tempted to satisfy their patients by "giving" them antibiotics. On the other hand, some of the studies showed that the employed patients are more likely to get an antibiotic prescription for acute bronchitis, because they make a pressure on a physicians believing wrongly that antibiotics would bring them back to their job sooner[19, 22, 23]. This effect was not reproduced in our study, probably reflecting different attitude to job of workers in transitional countries, like Serbia.

Several studies showed that years of medical training are inversely related to the frequency of prescribing antibiotics to the patients with acute bronchitis. The residents less frequently prescribe antibiotic for acute upper respiratory tract infections than the physicians without specialization [24]. Knowledge of primary care physicians in regard to treatment of acute bronchitis could be better, as reported by Ackerman et al. [25] who found that 31% of physicians believed that prescribing antibiotics for acute bronchitis was a standard of care. Probably prolonged medical training brings deeper insight to the nature and appropriate treatment of the diseases which are frequently encountered in the general practice. Besides, it is more probable that the prescribers with a higher level of professional training are acquainted with current treatment guidelines, and therefore less likely to prescribe drugs inappropriately.

Inappropriate prescribing of antibiotics to the patients with acute bronchitis was reported to be more frequent if physicians had more patient encounters per day. It was explained by lack of time during the working hours, so it was easier a physician to prescribe an antibiotic than to explain to the patient why it was not necessary [26, 27]. In our study the opposite was found, probably because an average number of patients per day was lower than in previous studies, letting other factors get involved, i.e., knowledge and experience of the attending physicians. The rate of patient encounters per prescriber is actually a composite indicator, which could be interpreted only if all details of local settings are known. It could be an indicator of prescribers' work overload due to insufficient staffing, but also of quality of care an individual prescriber offers to the patients, as he or she will have more patients who want to be cared for than other physicians. The first interpretation is more likely in Serbian settings, as understaffing is a serious problem at all levels of healthcare during the last decade.

Conclusion

Inappropriate prescribing of antibiotics to the patients with acute bronchitis is frequent phenomenon, although it is contrary to the recommendations of current therapy guidelines. Older patients are more likely to receive inappropriate antibiotic prescription, especially if their physician is without specialist training and has less patient encounters in his/her office daily.

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Received on July 31, 2017.
Revised on September 18, 2017.
Accepted on September 29, 2017.
Online First October, 2017.

ORIGINAL ARTICLE

UDC: 616-006:616.831-033.2
https://doi.org/10.2298/VSP161217145K

Whole brain irradiation with simultaneous integrated boost in treatment of oligometastatic brain disease

Zračenje celog mozga uz istovremeni integrisani dodatak doze kod oligometastatske bolesti mozga

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Abstract

Background/Aim. Brain metastases occur in 20%–30% of all patients with systemic cancer. We aimed at investigating whether patients with oligometastatic brain disease treated with whole brain radiotherapy (WBRT) and simultaneous integrated boost of brain metastases (SIB_{mets}) improved overall survival (clinical outcomes) compared with patients from the Radiation Therapy Oncology Group (RTOG) 9508 database, treated with WBRT and sequential stereotactic radiosurgery (SRS) boost. **Methods.** WBRT with SIB_{mets}, using the RapidArc (RA) (Varian Medical Systems, Palo Alto, CA) volumetric modulated arc technique (VMAT), was delivered to 15 patients with computed tomography/magnetic resonance imaging (CT/MRI) findings of 1–3 brain metastases with a diameter less than 40 mm for the largest lesion. Radiotherapy (RT) plans consisted of WBRT, with a prescribed dose of 20 Gy in 5 fractions, with SIB_{mets} which was also 20 Gy (gray units) in 5 fractions. **Results.** A group of 15 patients included 8 males and 7 females with the mean age of 56.3 years. Three patients were in the RTOG Recursive Partitioning Analysis (RPA) Class I and other 12 patients in RPA Class II. Four patients had one metastasis and 11 patients had two metastases. Calculated mean survival time (MST) was 7.49 ± 4.36 months with no statistically significant difference compared to RTOG 9508 results (MST = 6.5 months) ($p = 0.197$). The local control rate for 7 patients after three months was 85.7%. **Conclusion.** WBRT with SIB_{mets} and WBRT + SRS are clinically equivalent treatment options for the patients with oligometastatic brain disease. In comparison to the WBRT + SRS, the treatment by WBRT + SIB_{mets} technique reduces the treatment time and improves the patient's treatment comfort.

Key words:
brain; neoplasm metastasis; radiation oncology; radiotherapy; diagnosis, computer-assisted; radiotherapy, adjuvant; prognosis; mortality.

Apstrakt

Uvod/Cilj. Metastaze u mozgu se javljaju kod 20%–30% bolesnika sa sistemskom malignom bolešću. Cilj istraživanja bio je da se utvrdi da li bolesnici sa oligometastatskom bolešću mozga, tretirani zračenjem celog mozga (WBRT) u kombinaciji sa istovremenim ozračivanjem moždanih metastaza (SIB_{mets}), imaju poboljšano ukupno preživljavanje (klinički ishod) u poređenju sa bolesnicima iz *Radiation Therapy Oncology Group* (RTOG) 9508 baze podataka, tretiranim sa WBRT i sekvencijalnom stereotaktičnom radiohirurgijom (SRS) moždanih metastaza. **Metode.** Zračenje WBRT sa SIB_{mets} sprovedeno je volumetrijski modulisanom lučnom zračnom tehnikom (VMAT), pri čemu je zračenje celog mozga sprovedeno dozom 20 Gy u pet frakcija uz simultano zračenje metastaza mozga sa dodatnih 20 Gy u pet frakcija. Analizirano je 15 bolesnika sa prethodno verifikovanim metastazama u mozgu (od 1 do 3 metastaze) pomoću kompjuterizovane tomografije/magnetne rezonancije (CT/MRI), prečnika manjeg od 40 mm za najveće lezije. **Rezultati.** Petnaest bolesnika je bilo obuhvaćeno istraživanjem, osam muškaraca i sedam žena, prosečne dobi od 56,3 godine. Prema kriterijumima RTOG *Recursive Partitioning Analysis* (RPA), tri bolesnika su bila u klasi I, a 12 bolesnika u klasi II. Četiri bolesnika imala su jednu metastazu, a 11 bolesnika dve metastaze u mozgu. Izračunato srednje vreme preživljavanja (MST) bilo je 7.49 ± 4,36 meseci, bez statistički značajne razlike u poređenju sa rezultatima RTOG 4508 (MST = 6,5 meseci) ($p = 0.1975$). Stopa lokalne kontrole metastatske bolesti za sedam bolesnika nakon

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tri meseca bila je 85.7%. **Zaključak.** WBRT sa SIB_{mets} je klinički ekvivalentan tretmanu WBRT + SRS za pacijente sa oligometastatskom bolešću mozga. U poređenju sa WBRT + SRS, primena WBRT + SIB_{mets} tehnike zračenja skraćuje vreme lečenja i poboljšava komfor bolesnika.

Ključne reči:
mozak; neoplazme, metastaze; onkologija, radiološka; radioterapija; dijagnoza, kompjuter-asistirana; radioterapija, adjuvantna; prognoza; mortalitet.

Introduction

Brain metastases are the most common intracranial tumour. They occur in 20%–30% of all patients with metastatic disease [1]. The incidence is increasing due to the progress in cancer treatment (improved extracranial control and longer overall survival) and the increased utilization of modern imaging methods. The most common primary tumours responsible for brain metastases reported in adults are: lung cancer (16.3%–19.9%), melanoma (6.9%–7.4%), renal cell carcinoma (6.5%–9.8%), breast carcinoma (5.0%–5.1%) and colorectal carcinoma (1.2%–1.8%) [2]. For a long time, the treatment options were limited because surgical resection was not possible for the patients with multiple brain metastases, while chemotherapy had an effect only on a small group of patients with highly chemosensitive primary cancers. Without any treatment, prognosis was poor, while corticosteroid and antiepileptic treatment could control symptoms and prolong survival for a short period of time. On the other hand, ability to treat the patients with any number of brain metastases and regardless of the primary tumour histology, established radiotherapy (RT) as a cornerstone in the treatment of brain metastases. RT is an important palliative option for the patients with brain metastases since it alleviates symptoms, decreases the use of corticosteroids needed to control tumour-associated oedema and potentially improves overall survival [3, 4]. Definitive treatment options continue to evolve and include: surgery, chemotherapy and different radiotherapy techniques [whole brain radiotherapy (WBRT), stereotactic radiosurgery (SRS) and stereotactic radiotherapy (SRT)]. WBRT alone is the method of choice in the patients with the multiple brain metastases or lesions that are too large (diameter more than 40 mm) or inaccessible for surgery or SRS [4]. Usually, the dose of 20 to 30 Gy (gray units) is delivered to the whole endocranium in 5 to 10 fractions.

WBRT is also used in combination with surgery, SRS and SRT, to prevent the local recurrence or development of new brain lesions. Standard approach in the treatment of a single unresectable brain metastasis and oligometastatic brain disease (1–3 metastases) should be WBRT with SRS [5]. The procedure comprises a combination of WBRT and SRS performed as the separate procedures with 1–2 weeks interval in between.

During the last fifteen years, we have witnessed a rapid technical progress in the field of RT. The volumetric modulated arc technique (VMAT) allows the highly conformal intensity-modulated three-dimensional dose distributions to be delivered with one or two full rotations of the linear accelerator (Linac). With this technique it is possible to treat the patients with oligometastatic brain disease by irradiating the whole brain and metastases simultaneously with different doses.

Because of previously submitted data [5], we initiated a non-randomized prospective study attempting to investigate whether the patients with oligometastatic brain disease treated with WBRT and simultaneous integrated boost of brain metastases (SIB_{mets}) had an improved overall survival (clinical outcomes), compared to the Radiation Therapy Oncology Group (RTOG) 9508 database, which clearly showed benefits of combined approach in the patients treated with WBRT + SRS.

Methods

Fifteen patients with computed tomography/magnetic resonance imaging (CT/MRI)-confirmed brain metastases in the RTOG Recursive Partitioning Analysis (RPA) classes I and II were included in this study (Table 1) [6]. Every patient was presented and approved for brain RT by a multidisciplinary tumour board. The inclusion criteria were: age over 18, the CT/MRI findings of 1–3 brain metastases with a diameter less than 40 mm for the largest lesion, overall good performance status [the Eastern Cooperative Oncology Group (ECOG) ≤ 2] or the Karnofsky Performance Status (KPS) > 70. The non-inclusion criteria were: bad performance status (ECOG ≥ 3; KPS < 70), multiple brain metastases (≥ 4), metastases at a distance of less than 5 mm from optic chiasm, optic nerves or brainstem, solitary brain metastasis accessible for surgery or SRS and previous cranial RT. The exclusion criterion was treatment cancelation either to serious acute toxicity or underlying medical condition.

In our RT department, the patients with oligometastatic brain disease were treated with WBRT and SIB_{mets}. This is the Linac (Varian DHX)-based RT technique, using a frameless patient immobilization in combination with an image-guided radiotherapy (IGRT) and the VMAT technique. The patients were in a supine position with a Double Shell Positioning System (DSPS, Macromedics BV) and planning CT scans without intravenous contrast were obtained with a 1.25 mm slice thickness (Figure 1). This RT technique is hypofractionated, frameless (non invasive), while the treatment high precision is obtained by the IGRT techology.

Contrast-enhanced T2 sequences of co-registered diagnostic MRI scans were used for the target delineation. The whole brain planning target volume (PTV_{wb}) consisted of whole brain clinical target volume (CTV_{wb}) with symmetrical 2 mm margin. Boost planning target volume of the metastases (PTV_{boost}) was derived by adding a 2 mm margin to gross tumor volume (GTV_{boost}), (Figure 2). This margin takes into account the possible residual positioning inaccuracies using an online Cone Beam Computed Tomography (CBCT) setup protocol.

Table 1

Patients characteristics

	All patients	Male	Female
Number of patients, n (%)	15 (00)	8 (53.3)	7 (46.7)
Age (years), n (%)			
< 65	12 (80)	6 (40)	6 (40)
≥ 65	3 (20)	2 (13.3)	1 (6.7)
median age	56.3	60.4	51.6
Primary tumour origin			
lung cancer	6 (40)	5 (33.3)	1 (6.7)
breast cancer	3 (20)	-	3 (20)
duplex carcinoma	3 (20)		
rectal + lung cancer	1 (6.7)	1 (6.7)	-
sygmoid colon + renal cancer	1 (6.7)	1 (6.7)	-
oesophageal + lung cancer	1 (6.7)	1 (6.7)	-
rectal cancer	1 (6.7)	-	1 (6.7)
ovarian cancer	1 (6.7)	-	1 (6.7)
unknown primary	1 (6.7)	-	1 (6.7)
histology (n)			
adenocarcinoma	6	4	2
planocellulare	2	2	-
ductal	2	-	2
clear cell (renal)	1	1	-
adenocarcinoma papillare (ovarian)	1	-	1
neuroendocrine large cell (lung)	1	1	-
data non available	4	2	2
RPA class, n (%)			
1	3 (20)	1 (6.7)	2 (13.3)
2	12 (80)	7 (46.7)	5 (33.3)
ECOG, n (%)			
0–1	12 (80)	6 (40)	6 (40)
2	3 (20)	2 (13.3)	1 (6.7)
Metastases, n (%)			
brain only	8 (53.3)	5 (33.3)	3 (20)
brain and one other extracranial site	4 (26.7)	3 (20)	1 (6.7)
brain and ≥ 2 extracranial sites	3 (20)	-	3 (20)
Number of brain metastases			
1	4 (26.7)	2 (13.3)	2 (13.3)
2	11 (73.3)	6 (40)	5 (33.3)
Size of the metastases (n)			
< 2 cm	13		
2–3 cm	10		
3–4 cm	4		

Recursive Partitioning Analysis (RPA) Classes I and II were included according to Gaspar et al. [6]; ECOG – Eastern Cooperative Oncology Group.

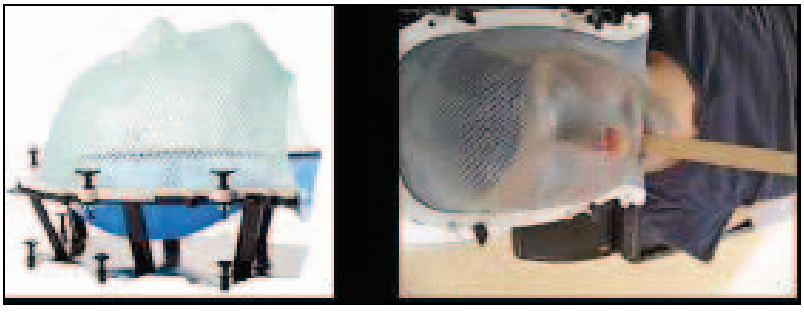

Fig. 1 – Double Shell Positioning System (Macromedics BV).

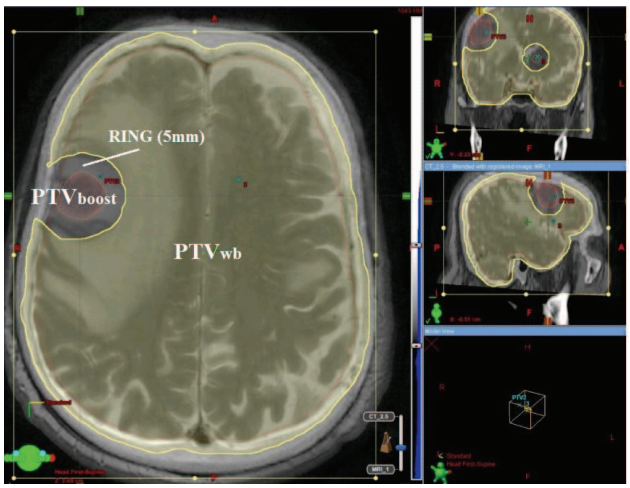

Fig. 2 – Whole brain planning target volume (PTVwb – yellow) and boost planing target volume of the metastases (PTVboost – pink).

Minimal accepted criteria to the PTV_{wb} and the PTV_{boost} was that 95% isodose covers 100% of both PTV volumes. The prescribed fraction dose was 4 Gy to PTV_{wb} and 8 Gy to PTV_{boost}, respectively [7]. There was no maximum dose limit for the brain metastasis (Figure 3).

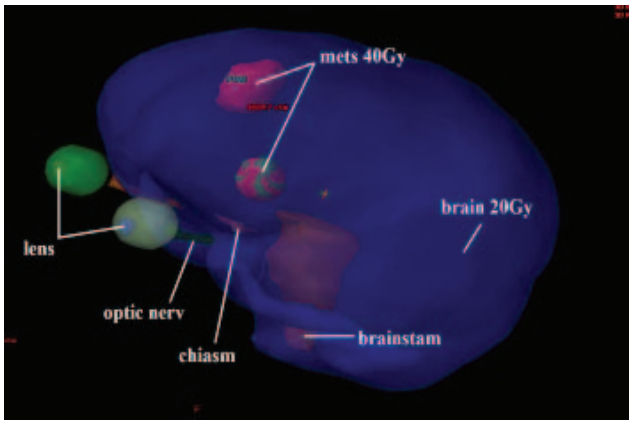

Fig. 3 – Organs at risk and prescribed dose on PTVwb and PTVboost. For abbreviations see under Fig. 2.

The composite RT plans were generated for 15 patients. The RT plans consisted of WBRT, with prescribed dose of 20 Gy in 5 fractions and SIB_{mets} which was also 20 Gy in 5 fractions. The cumulative dose received by the brain metastases was consequently 40 Gy in 5 fractions. The treatment plans were generated by 6 megovoltage (MV) photons, the RapidArc (RA) technique, two full arcs, multileaf collimator (MLC) with a leaf width of 5 mm (Varian-Millennium 120 MLC) and a collimator rotation of ± 30°. All final dose calculations were performed with the Varian Eclipse TPS, version 10.0. The planning algorithm, Acuros XB, used progressive sampling optimization by simultaneously changing the shape of the treatment aperture, dose rate (max 600 MU/min – monitor unit/min) and rotation speed of the gantry. We have also calculated the conformity index ($CI_{95\%}$), defined as ratio of 95% isodose volume and PTV_{boost} volume. $CI_{95\%}$ should be as close as possible to 1.

In the SRS treatment a dose of 18–25 Gy was usually prescribed to the PTV_{boost}, corresponding to a biologically effective dose (BED) 50.4–87.5 Gy_{10} and a biologically equivalent dose (EQD_2) 42–72.9 Gy_{10}, calculated using the α/β ratio of 10 for tumour tissue. Minimum dose of 95% of 40 Gy in 5 fractions to the PTV_{boost} volume with RA corresponds to a (BED) 66.9 Gy_{10}, (EQD_2) 55.7 Gy_{10} [7].

Online CBCT was performed before every fraction, after orthogonal kV-kV imaging. Usually, we repeat CBCT after delivered fraction to determine the intrafraction motion. The GTV_{boost} to PTV_{boost} margin should account for both: translational and rotational setup uncertainties. Translational setup errors were eliminated by the patient repositioning, according to pretreatment imaging (presence of physician is required). According to the three rotations (Roll-Pitch-Rtn) and the distance between the isocenter and the center of further metastases, we calculated the rotational setup error to determine the margin between GTV_{boost} and PTV_{boost}.

All calculated VMAT plans were delivered on Linac and the dosimetry verification was performed before the first treatment by theVarian Portal Dosimetry (EPID Portal Vision IDU20, aSi1000) or Delta4 (ScandiDos AB). The criteria were that 95% of pixels passed with a dose tolerance 2% of reference values and distance to agreement (DTA) 2 mm, Gamma (2%, 2 mm).

WBRT with SIB_{boost} was delivered to 15 patients from February 2014 to January 2016. The last patient data was collected on 1st September 2016. The accurate date of patient's time of death was collected in a direct communication with the patient's primary oncologist or close family members.

The primary outcome was overall survival in the patients with oligometastatic brain disease. The secondary outcomes were the radiographic tumour response and local control rates. Survival was measured from the start of the RT treatment until death or 1st September 2016. Survival was estimated with the Kaplan-Meier method [8]. We have also performed the single sample Student`s *t*-test (one tail hypothesis test) to find out the test statistics. For the evaluation of radiographic tumour response and local control rates, the initial MRI findings and MRI findings on follow-up scans were measured and compared.

The follow-ups were scheduled at a three-month interval, including the clinical evaluation and control MRI. The radiographic tumour response and local control rates were classified as a complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD). CR was defined as a total radiographic disappearance of all lesions. PR was defined as at least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters. PD was defined as at least a 20% increase in the sum of diameters of target lesions, taking as a reference the smallest sum in the study (this includes the baseline sum if that was the smallest in the study). In addition to the relative increase of 20%, the sum also had to demonstrate an absolute increase of at least 5 mm. The appearance of one or more new lesions was also considered a progression. SD was defined as neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD [9].

Results

The mean age of 15 evaluated patients (8 males and 7 females) was 56.3 years, standard deviation (StD) of 13.2 years. Distribution of primary tumour diagnoses was: lung cancer 6 patients (40%); breast cancer 3 patients (20%); rectal cancer 1 patient (6.7%); ovarian cancer 1 patient (6.7%). Three patients had two different carcinomas at the same time (rectal and lung carcinoma, colon and renal carcinoma, esophageal and lung carcinoma), while one patient had unknown primary carcinoma.

Based on the Kaplan-Meier method, the calculated mean survival time (MST) was 7.49 ± (StD) 4.36 months (Figure 4) with no statistically significant difference in comparison to RTOG 4508 results (MST = 6.5 months) onetailed single sample Student's *t*-test, $p = 0.197$. Seven patients came for the first follow-up evaluation, while only 3 patients came for the second check-up 6 months after the treatment completion. The local control rate after 3 months was 85.7% (CR 14.3%; PR 4.9%, SD 28.6%) (Figure 5). After 6 months one patient was still in CR, one was in PD because of the development of new brain metastases, while one patient was in SD.

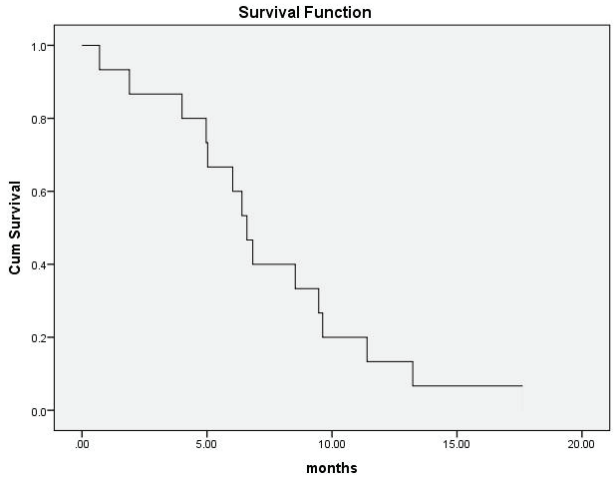

Fig. 4 – The Kaplan-Meier overall survival time curve for 15 patients.

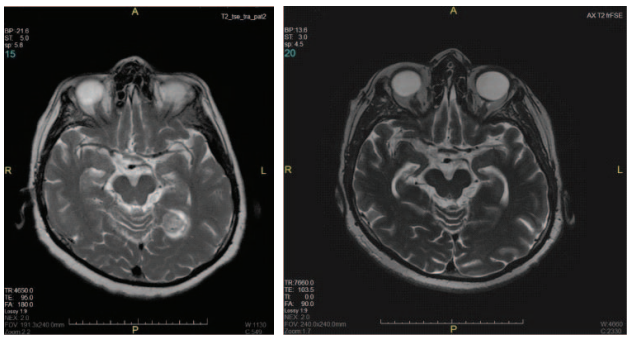

Fig. 5 – (left) T2 sequences of baseline magnetic resonance imaging (MRI) and, (right) follow-up MRI after 6 months showing a complete response (CR).

Range of GTV_{boost} was from 0.3 cm^3 to 23.5 cm^3 (StD 7.1 cm^3). Consequently, PTV_{boost} volumes were in range from 1.2 cm^3 to 32.8 cm^3 (StD 10.2 cm^3). The conformity index for the total PTV_{boost} volumes was 1.09 (StD 0.09). The data for the mean volumes of CTV_{wb} and PTV_{wb} were 1,471 cm^3 (StD 153 cm^3) and 1,695 cm^3 (StD 153 cm^3) respectively. The conformity index for PTV_{wb} was 1.155 (StD 0.31).

The mean value of monitor units needed to deliver the fraction doses of 8 Gy was 1,986 (StD 80). An example of an integrated RA plan is shown in Figure 6. The dosimetric analysis of the composite RA plans showed excellent coverage both of PTV_{wb} and PTV_{boost}, with the mean volumes receiving at least 95 % of the prescribed dose. The maximum dose, which was in all cases located within the PTV_{boost}, had a mean value of 113.4% (107.7–126.4%) and StD 4.3%. The doses to critical organs at risk (OAR) were evaluated by the use of dose volume histograms (DVH) (Figure 7). As a result of modulation dose in the 5 mm ring around PTV_{boost} area had a steep dose gradient (Figure 8). The composite plans showed a dose decrease outside the brain metastases, inside the ring around PTV_{boost}, from 38 Gy to 28 Gy, which meant that the dose falloff was at least 2 Gy/mm. The pretreatment measured dose distribution generally agreed well with the calculated dose from the treatment planning system. The mean gamma, averaged for all measured plans for the double arc, was 0.345 (SD 0.038). Area gamma was 97.6% (StD 1.3%).

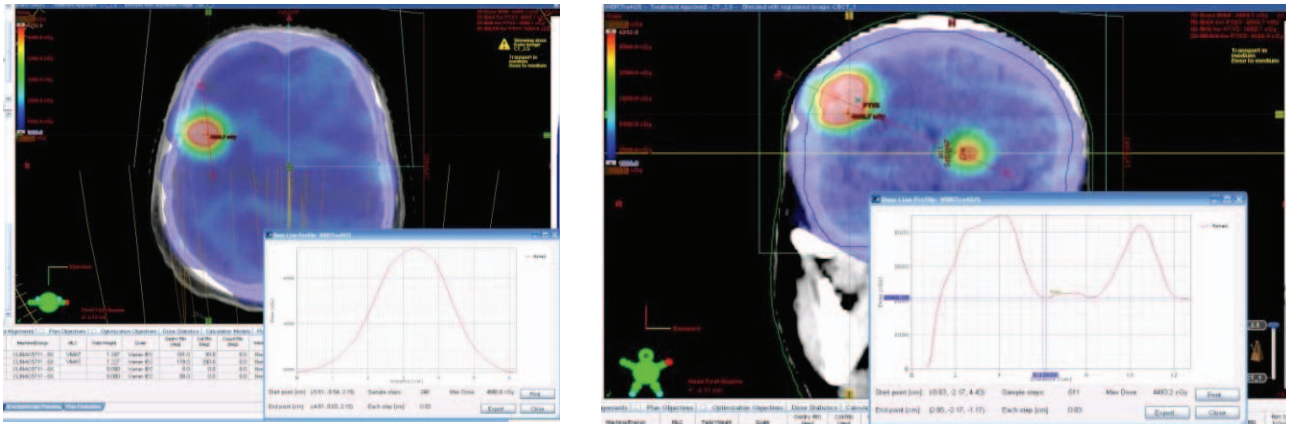

Fig. 6 – RapidArc treatment plan for particular patient with two metastases.

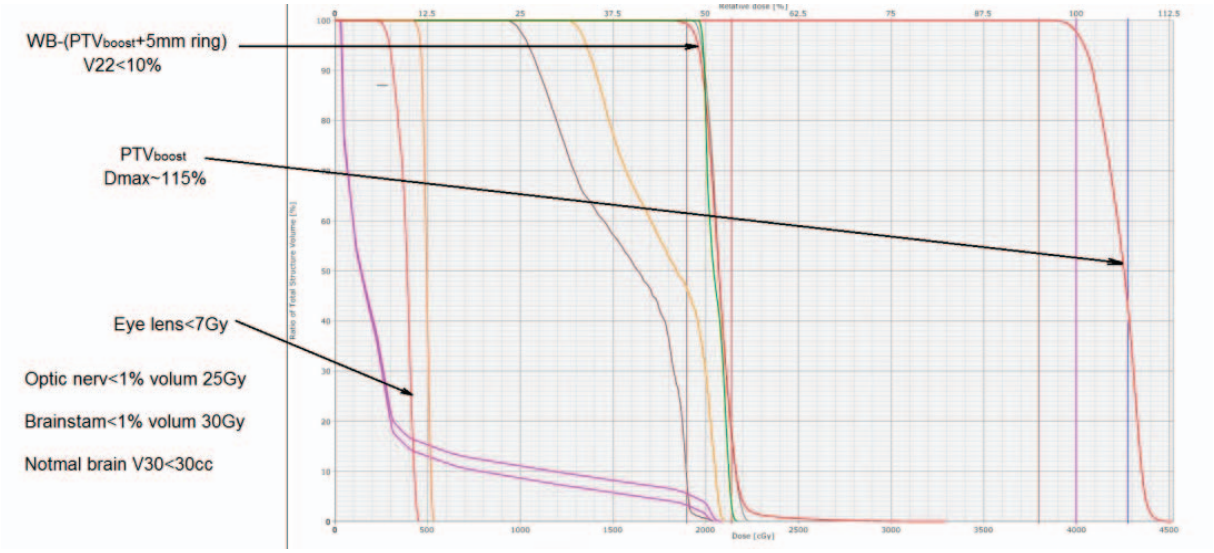

Fig. 7 – Dose volume histogram (DVH) for target volumes and organs at risk.

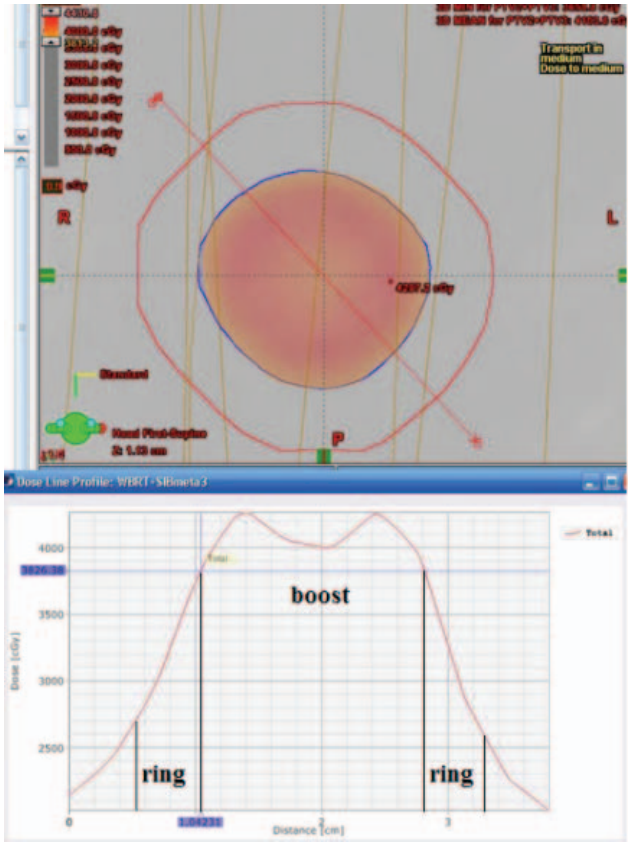

Fig. 8 – Steep dose gradient inside the ring area and dose profile inside PTVboost and a 5 mm ring around it. For abbreviation see under Fig. 2.

According to the pretreatment CBCT, our average values for Roll-Pitch-Rtn angles were 0.5° (StD 0.5°) and a distance from isocenter 59 mm (StD 17 mm), which leads to spatial distances of 0.9 mm. It meant that we were within a 2 mm GTV_{boost} to PTV_{boost} margin. All the rotations exceeding limitation were corrected by reapplying the mask and repeating the CBCT to ensure that the correct position was obtained.

Despite using the noninvasive immobilization device, the treatment delivery times (”in-room”), varied from approximately 20–25 minutes for each fraction. The beam-on time was 3–4 min, which consequently decreased the risk of intrafractional positional shifts of the patient within the fixation device.

Discussion

WBRT with SIB_{mets}, SRS and surgery are adequate treatment options for solitary brain metastasis. Because no randomized trials have been conducted to assess differences between SRS and surgery, the choice is purely based on a clinical judgment. When used in a combination with surgery, WBRT reduced the incidence of recurrence of brain metastases down from 70% in the surgery alone group to 18% in the surgery + WBRT group [10]. For SRS alone, the one-year recurrence rate is 76.4%, while in WBRT + SRS group it is 46.8% [11].

Several randomized trials were conducted with a purpose to evaluate the survival benefit of addition of WBRT to surgery, or SRS over WBRT alone (Table 2). Two of three evaluated trials showed that combined approach of WBRT + surgery, or SRS improved treatment outcomes for the patients with single metastasis compared to WBRT alone [12–14]. Also, the combination of local irradiation of oligometastatic disease and WBRT represents a superior treatment modality in terms of improving local tumour control [15, 16].

RTOG 9508 showed an overall mean survival advantage for patients with oligometastatic brain disease, treated with WBRT+SRS versus WBRT alone (MST 6.5 months versus 5.7 months; $p = 0.1356$). This study definitely indicates that WBRT + SRS provides survival benefit to patients with single metastasis (6.5 months versus 4.9 months; $p = 0.0393$) and because of the improved performance in the patients who received adjuvant SRS after WBRT, we should consider WBRT + SRS when treating the patients with oligometastatic brain disease [5].

Table 2

Literature review of treatments for brain metastases

Authors	Treatment modality	Number of patients	Mean survival time (months)	Statistical significance
Patchell et al. 1990 [12]	WBRT	23	3.5	$p < 0.01$
	WBRT + Surgery	25	9.2	
Noordjik et al. 1994 [13]	WBRT	31	6	$p = 0.04$
	WBRT + Surgery	32	10	
Mintz et al. 1996 [14]	WBRT	43	6.3	$p = 0.24$
	WBRT + Surgery	41	5.6	
Andrews et al. 2004 [5]	WBRT	94	4.9	$p = 0.04$
	WBRT + SRS	92	6.5	
Kondziolka et al. 1999 [17]	WBRT	14	7.5	$p = 0.22$
	WBRT + SRS	13	11	
Aoyama et al. 2006 [11]	SRS	60	7.5	$p = 0.42$
	WBRT + SRS	60	8	

WBRT – whole brain radiotherapy; SRS – sequential stereotactic radiosurgery.

Our calculated mean survival time (MST) of 7.49 months ($p = 0.197$) in comparison to RTOG 9508 MST 6.5 months, showed us that for the patients with oligometastatic brain disease WBRT + SIB_{mets} was clinically equivalent treatment option to WBRT + SRS. Even though our sample of 15 patients was not sufficiently large to provide a statistical proof of the significantly better prognosis due to the big standard deviation, assigning the patients to subgroups based on the number of metastases or RPA classes. Also, it was not possible to do the follow-up on more patients due to their low response and refusing to travel long distances to undergo the diagnostic scans needed for the follow-up evaluations.

The evaluation of dosimetric parametes for radiotherapy treatments of our patients coincide to the same published data, which confirms that, in comparison to WBRT + SRS, VMAT WBRT + SIB_{mets} is at least efficient radiotherapy technique to other radiotherapy approaches [5, 7, 17]. At the same time, by using the VMAT WBRT + SIB_{mets} technique it is possible to achieve the same or better radiobiological effects in comparison to WBRT + SRS. Also, the reduced number of fractions combined with rigid frameless fixation decrease the risk of intrafraction positional shifts and treatment time while improving the patient comfort during the treatment.

Conclusion

WBRT + SRS and VMAT WBRT + SIB_{mets} are clinically equivalent treatment options for the patients with oligometastatic brain disease. In comparison to the WBRT+SRS, treatment by the VMAT WBRT + SIB_{mets} technique reduces the treatment time and improves the patient treatment comfort.

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Received on December 17, 2016.
Revised on September 25, 2017.
Accepted on September 29, 2017.
Online First October, 2017.

ORIGINAL ARTICLE

UDC: 616.36-006-08
https://doi.org/10.2298/VSP170721152J

Embolizations of the hepatic tumors - two-year single center experience

Embolizacije tumora jetre – dvogodišnje iskustvo jednog centra

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Abstract

Background/Aim. Transcatheter arterial chemoembolization (TACE) and portal vein embolizations (PVE) are established methods of treatment of patients with hepatic tumors. The aim of the study was to present our experience in the treatment of liver tumors with embolization as a preliminary treatment for surgery or a part of palliative treatment. **Methods.** The analysis included 29 patients who had undergone 34 embolizations. **Results.** TACE was performed in 26 cases with hemangiomas in the unfavorable location, or mass effect and inoperable malignant tumors both primary and metastatic. PVE was performed in 8 cases with primary liver tumors and colon liver metastases.All included patients presented inoperable hepatic tumors. TACE was carried out in the patients with hepatocellular carcinoma (n = 1), cholangiocarcinoma (n = 1), metastatic tumor (n = 8), and hemangioma (n = 16), while PVE in the patients with cholangiocarcinoma (n = 2), metastatic tumor (n = 5) and neuroendocrine tumor (n = 1). The embolization was followed by surgery in the 5 PVE patients and 6 TACE patients. The postembolization syndrome was observed in 7 subjects. Death due to cancer progression occurred in the 4 PVE patients and 7 TACE patients. One patient died during TACE due to hemorrhagic shock. **Conclusions.** Right PVE and selective TACE are efficient for preliminary preparation of patients with healthy hepatic parenchyma for major liver resections, but the patients with liver cirrhosis require careful assessment. In the patients with hemangioma, embolization allows to avoid surgical treatment by reducing the lesion mass, or the extent of hepatic resection. The preliminary results of arterial embolizations with bleomycin, leading to tumor reduction in cases of giant liver hemangiomas are promising.

Key words:
liver neoplasms; embolization, therapeutic; digestive system surgical procedures; bleomycin.

Apstrakt

Uvod/Cilj. Transkateterska arterijska hemoembolizacija (TAHE) i embolizacija portne vene (EPV) su ustanovljene metode u lečenju tumora jetre. Cilj studije bilo je prikazivanje našeg iskustva u lečenju tumora jetre, bilo kao preliminarno lečenje pre hirurškog zahvata, bilo kao deo palijativnog lečenja. **Metode.** Analiza je uključila 29 bolesnika kojima su učinjene 34 embolizacije. **Rezultati.** TAHE je učinjena kod 26 bolesnika sa hemangiomima na nepovoljnoj lokalizaciji ili kao redukcija kod inoperabilnih malignih tumora, bilo primarnih ili metastatskih. EPV je učinjena kod osam bolesnika sa primarnim tumorima jetre i metastazama u jetri iz kolona. Kod svih su tumori jetre bili inoperabilni. TAHE je učinjena kod jednog bolesnika sa hepatocelularnim karcinomom, jednog sa holangiokarcinomom, kod osam sa metastatskim tumorom, i kod 16 bolesnika sa hemangiomom. EPV je učinjena kod dva bolesnika sa holangiokarcinomom, pet bolesnika sa metastatskim tumorom i jednog sa neuroendokrinim tumorom. Kod pet bolesnika EPV embolizacija prethodila je hirurškom tretmanu, a TAHE kod šest bolesnika. Postembolizacijski sindrom je praćen kod sedam bolesnika. Smrt zbog progresije karcinoma nastupila je kod četiri EPV bolesnika i 7 TAHE bolesnika. Jedan bolesnik umro je tokom TAHE zbog hemoragijskog šoka. **Zaključak.** Uspešna desna PVE i selektivna TAHE su efikasne kod preliminarne pripreme bolesnika sa zdravim parenhimom jetre za opsežne resekcije jetre, ali bolesnici sa cirozom jetre zahtevaju pažljivu procenu. Kod bolesnika sa hemangiomom, embolizacija omogućava izbegavanje hirurškog zahvata zbog hirurške redukuje tumorske mase ili smanjuje opsežnost hirurškog zahvata. Preliminarni rezultati arterijske embolizacije bleomicinom pokazuju obećavajuće rezultate kod gigantskih hemangima jetre.

Ključne reči:
jetra, neoplazme; embolizacija, terapijska; hirurgija digestivnog sistema; procedure; bleomicin.

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Introduction

Embolization is a method used in the treatment of liver tumors as an alternative to surgical treatment as well as for a preparation for major liver resections [1]. In the 4th Military Clinical Hospital in Wroclaw, embolizations with the closure of both the hepatic arteries and portal vein branches have been performed since July 2013. This procedure is reserved for the patients who do not qualify for surgery due to a large tumor, hazardous (particularly parahilar) location, or poor overall health status while meeting conditions for embolization. By blocking the tumor blood supply, arterial embolization selectively cuts off the supply of nutrients and oxygen to the tumor tissues.

Embolization procedure consists of introducing a fine catheter along the arteries into the close vicinity of the tumor and delivering a substance that causes the closure of the arterial vessel. In contract to healthy liver parenchyma, the tumor cells receive the nutrients from the hepatic artery rather than from the hepatic vein vessels; thus, transcatheter arterial chemoembolization (TACE) was introduced to administer a chemotherapeutic agent followed by the vessel closure and radioembolization. The most frequent radiotherapeutic agent used in TACE is yttrium-90, which, delivered in microspheres, undergoes radioactive decay to release energy by destroying the tumor cells [2, 3].

The portal vein embolization (PVE) is a procedure involving the transcutaneous punctures of the branches of the right (less commonly the left) branch of the portal vein followed by an introduction of the vessel-closing agent. This is aimed at blocking blood from being supplied via the portal vein into a hepatic lobe to induce secondary hypertrophy of the other hepatic lobe. This allows for the major resection procedures being performed on the initially inoperable liver tumors. Hepatic hypertrophy increases the efficacy of the part of hepatic parenchyma being left after the resection; thus, PVE is a procedure that prepares the patient for major resections and enables the remaining part of the liver to function properly. Optimum hepatic hypertrophy is achieved 2–4 weeks after the procedure in healthy hepatic parenchyma and 6–8 weeks after the procedure in liver cirrhosis of steatosis.

The aim of the study was to present the embolization as a method for a treatment of inoperable liver tumors and a part of palliative treatment in large or numerous nodular liver lesions in the patients disqualified from surgery, with lesions in parahilar location or as a preliminary treatment for surgery of large focal liver lesions within a single lobe.

Methods

The study included 34 embolizations performed in 29 patients (14 male and 15 female patients) in the 4th Military Clinical Hospital in Wroclaw, Poland between July 2013 and December 2015. The mean age of all patients was 55.5 years. At the time of the procedure, the youngest patient was a 39-years-old male patient with an extensive hemangioma within the right hepatic lobe treated with arterial embolization procedure. The oldest patient was an 83-years-old male patient subjected to two arterial embolization procedures due to inoperable hepatic metastases of colon cancer.

Among analyzed embolizations, 26 procedures were TACE and 8 PVE. Indications for TACE included the large benign liver tumors (hemangiomas, focal nodular hyperplasias) with unfavorable, i.e., parahilar location or presenting with the mass effect as well as the large inoperable malignant tumors within the liver both primary and metastatic. TACE were also performed in the patients who were disqualified from the surgical treatment due to their overall health condition, numerous comorbidities, or the presence of other contraindications for general anesthesia. The embolizations were not performed in the patients with hepatic abscesses or cysts. TACE may be carried out periodically depending on the expected outcomes. Indications for PVE included large or numerous malignant tumours within a single hepatic lobe that, if resected without prior embolization, would lead to postoperative liver failure due to the insufficient amount of liver parenchyma being left after the surgery.

All procedures were followed in accordance with the ethical standards and with the Helsinki Declaration of 1964 and later versions. The informed consent was obtained from all the patients included in the study. The patients' medical records were analyzed retrospectively. The data were presented as means and percentages.

Results

In the study, the liver embolizations were performed in the patients with the inoperable hepatic tumors: 16 in benign and 18 in malignant. The benign lesions included hemangiomas, while the malignant lesions included hepatocellular carcinoma (HCC) – cases secondary to post-hepatitis C virus (HCV) and post-hepatitis B virus (HBV) inflammation cirrhosis; cholangiocarcinoma (CC); neuroendocrine tumors and colon cancer metastases. Three patients had to undergo repeated embolizations due to the extent of nodular lesions. Of these, one patient underwent TACE procedure 4 times as a staged treatment of extensive hemangiomas within both liver lobes. The patient was qualified for the surgery that involved the resection of liver segments 2, 3, 4, and 5. Lipiodol, polyvinyl alcohol (PVA) and metal springs were most often used as embolization agents. In 13 cases, bleomycin was used. Cases qualified for PVE included 2 patients diagnosed with HCC, one patient with a neuroendocrine tumor and 5 patients with hepatic metastases of colon cancer.

Right PVE was performed in 8 patients; 5 of these patients were subsequently subjected to the surgical treatment (Figures 1–3), while the remaining 3 underwent the embolization procedure as part of palliative treatment. Palliative TACE was performed in 6 cancer patients. In 10 patients with hemangiomas, TACE resulted in the reduction in the lesion size allowing for the abandonment of surgical treatment. Surgical procedures were performed after TACE in 6 patients. The liver failure symptoms developed after right-sided hemihepatectomy in 2 patients with post-inflammatory cirrhosis after the HBV infection and type II diabetes (following right PVE).

Table 1

The characteristics of the study group and the results of the treatment

Characteristics of the patients	Type of embolization	
	PVE (n = 8 cases)	TACE (n = 26 cases)
Mean age of patients (years)	63.3	47.7
Diagnosis	2 HCC, 5 metastases of colon cancer, 1 neuroendocrine tumor	1 HCC, 7 metastases of colon cancer, 1 neuroendocrine tumor metastasis, 16 hemangiomas, 1 CC
Mean duration of hospitalization (days)	5.5	4.5
Number of patients undergoing surgery	5 surgeries: 3 hemihepatectomies 1 laparotomy (biopsy) 1 laparotomy (biopsy and RTA)	6 surgeries: 4 cancer cases – laparotomy (biopsy) 2 hemangioma cases – resection procedures
Deaths (n)	4 due to cancer progression	1 perioperative 7 due to cancer progression

PVE – portal vein embolization; TACE – transcatheter arterial chemoembolization; CC – cholangiocarcinoma; HCC – hepatocellular carcinoma; RTA – radio-frequency thermoablation.

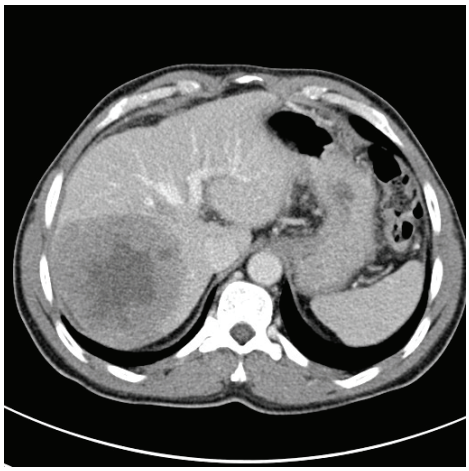

Fig. 1 – The patient with hepatocellular carcinoma before the portal vein embolization. Computed tomography scan shows a large hypodense and heterogenous tumor in the segments VII and VIII and partially in the segments V and VI of the right hepatic lobe, measuring 88 × 90 × 90 mm, with necrosis in the central part.

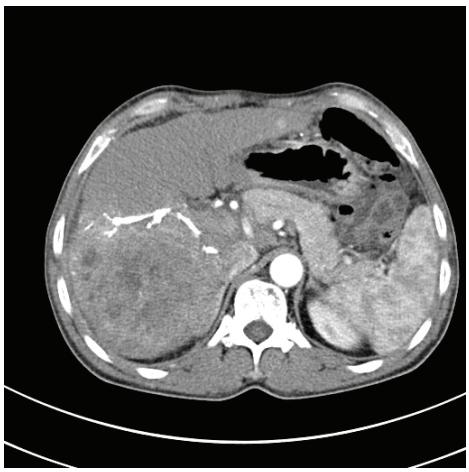

Fig. 2 – Computed tomography scan shows the reduced size of the hepatic tumor of the same patient after the procedure of portal vein embolization.

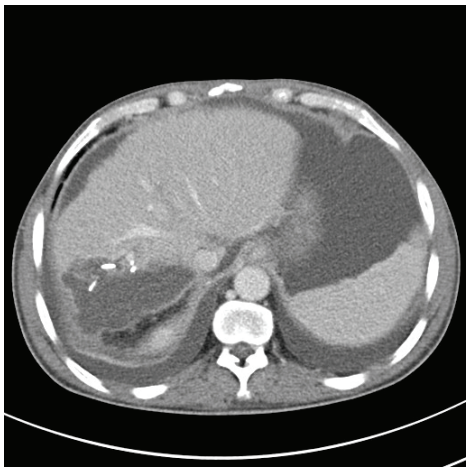

Fig. 3 – The same patient after the right hemihepatectomy. Computed tomography scan shows the irregular and encysted fluid collection in the tumor bed, measuring 80 × 52 × 42 mm, of 20 Hounsfeld units (HU) density, suggesting a postoperative inflammation.

Deaths occurred in 12 patients: one was due to perioperative hemorrhagic shock and 11 were due to the progression of cancer several months after the embolization. The postembolization syndrome was observed in 7 subjects and manifested as transient abdominal pain with isolated episode of increased body temperature up to 38°C observed during the hospitalization. The symptoms resolved after administration of analgesic and antipyretic drugs. No abscesses or necrotic foci requiring surgical interventions were observed. Table 1 presents characteristics of the study group and results of the treatment.

Discussion

In most countries, 5-year survival from liver cancer is low (below 20%) indicating that most patients are diagnosed when they are inoperable [4]. Additionally, primary liver and biliary cancers are very aggressive tumors which are the second leading cause of cancer death worldwide. The incidence

of those cancers decreases in Europe due to the decline of seroprevalence of hepatitis B virus (HBV) as well as targeted screening and treatment of the HCV [5]. In Poland, chronic HCV affects about 200,000 individuals, but very few of them are aware of the infection, and even fewer are treated [6]. The present study included only the inoperable liver tumors which were treated to enable surgery or as a part of palliative treatment. All 3 cases of HCC were associated with underlying hepatitis which is a risk factor for the poor prognosis.

Surgical resection is a gold standard for hepatic malignancies, but a majority of patients are diagnosed in inoperable state, thus embolization plays an important role in the treatment. The transarterial embolization is considered a palliative therapy for multifocal and large malignant tumors. The aim of this procedure is to deliver a chemotherapeutic drug and/or embolizing agent into the tumor, causing its necrosis. Data from the relevant literature indicates that better results are obtained in smaller lesions. Miraglia et al. [7] performed TACE in the patients with HCC and obtained complete necrosis in 68% of patients with lesion between 4.1–5.0 cm, 50% of patients with lesion between 5.1–6.0 cm, and only 13% of patients with lesions over 6 cm. In hemangioma, TACE may be used as alternative to surgery, or with the aim to reduce the tumor size prior to surgery. Sun et al. [8] reported a significant decrease in the tumor size from 11.24 to 7.60 cm six months after embolization. In the present study, surgery was performed in 6 (23.08%) patients who had undergone TACE. This type of embolization enabled surgery in 4 cancer patients, diminished extent of the resection by reducing the mass of hemangioma in 2 cases, and allowed to avoid surgical treatment in 14 hemangioma patients.

The aim of PVE is to induce regrowth of properly functioning liver parenchyma prior to a planned resection of a hepatic tumor. In case of malignant lesions, PVE may be combined with TACE. Disadvantage of this procedure is the delay of radical treatment increasing the tumor growth and the possibility of the lack of liver hypertrophy. The patients with primarily unresectable tumors require special approach because primary resectability of those tumors is limited mainly due to insufficient future liver remnant volume (FRLV). Risk factors such as chemotherapy, steatosis, diabetes mellitus, cholestasis and cirrhosis determine the development of the post-hepatectomy liver failure. Previous studies suggest that FRLV of above 20% is safe in healthy livers, above 30% in steatosis or during chemotherapy and above 40% in cirrhosis [9]. Many researchers create strategies that help to increase the FRLV pre-operatively to ensure the proper liver function after surgery. Embolization is one the methods which decrease the rate of hepatic complications. It is recommended when FRLV drops below 40% in the liver affected by a disease. [9–11] Volumetry of the liver is assessed directly before and 3–4 weeks after the embolization. Obtained hypertrophy correlates with frequency of liver complications, hepatic failure, length of hospitalization and mortality [12]. Extended hepatectomy may result in presentation with liver failure symptoms due to reduced mass of liver which is insufficient to maintain normal liver function. Clinical manifestation of liver insufficiency after extended hepatectomy is known as small for size syndrome (SFSS). Recent studies suggest that it is determined not only by FRLV but also by the hemodynamic parameters of the hepatic circulation [13–15]. In the present study, 2 out of 3 subjects with HCC, hepatic cirrhosis, and diabetes presented with liver failure symptoms after hemihepatectomy, despite meeting the 40% threshold of FRLV before surgery. This indicates that further research is required in the scope of the pathogenesis of SFSS which would allow surgeons for better qualification of the patients for liver surgery.

The presence of liver metastases in the patients with colon cancer determines poor prognosis. The surgical resection improves survival, but it may be impossible in cases with large lesions. The surgical removal of colorectal liver metastases improves survival in comparison with nonsurgical treatment (5-year overall survival rate 47% vs 6%). Repeated surgery has similar survival rate to surgical removal [5]. The present study included 5 cases of metastases of colon cancer subjected to PVE and 7 subjected to TACE. The majority of those embolizations were given to treat liver metastases for palliative effects.

Conclusion

The right portal vein branch or selective arterial embolization is an efficient method of preliminary preparation of patients with healthy hepatic parenchyma for major liver resections. No hepatic hypertrophy suitable for the proper functioning of parenchyma preserved after the resection was obtained in the patients with liver cirrhosis. In case of hemangiomas, the embolization allows to avoid surgical treatment by reducing the lesion mass, or to reduce the extent of resection procedures. The preliminary results of arterial embolizations with bleomycin leading to tumor reduction in cases of giant liver hemangiomas are very promising.

Acknowledgments

This study was not funded by any grants.

Conflict of interest

The authors declare no conflict of interest in this work.

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Received on July 21, 2017.
Revised on September 26, 2017.
Accepted on October 2, 2017.
Online First October, 2017.

ORIGINAL ARTICLE

UDC: 618.11:616-008.9]:616.12-037
https://doi.org/10.2298/VSP170504158B

Association of vascular and inflammatory markers with metabolic disorders in women with polycystic ovary syndrome

Udruženost vaskularnih i inflamatornih markera metaboličkih poremećaja kod žena sa sindromom policističnih jajnika

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Abstract

Background/Aim. The prevalence of metabolic disorders, obesity and insulin resistance in women with polycystic ovary syndrome (PCOS) occur early in life and places this group at risk of cardiovascular disease. Hyperhomocysteinemia and increased C-reactive protein (CRP) activity have an effect on promoting atherosclerosis. This study was designed to evaluate whether high sensitivity (hs-CRP) and homocysteine (Hcy) are elevated in PCOS and to elucidate their possible relation to obesity, insulin resistance, or metabolic changes usually present in women suffering from PCOS. **Methods.** Serum concentration of hs-CRP and plasma levels of Hcy were evaluated in 73 PCOS women and 43 healthy women, together with clinical, anthropometric and hormonal parameters. **Results.** The mean of body mass index (BMI), waist circumference (WC), waist to hip ratio and mean concentration of luteinizing hormone (LH), testosterone, androstenedione, free androgen index, fasting insulin, homeostatic model assessment of insulin resistance (HOMA- IR), hs-CRP and Hcy were significantly higher in PCOS women compared to age-matched healthy women. There was a positive correlation between hs-CRP and BMI, WC, insulin, triglycerides ($p < 0.001$) and significant negative correlation with LH, sex hormone binding protein (SHGB), HOMA-IR, high density lipoprotein cholesterol (HDL-C) ($p < 0.001$). The Hcy concentration had a significant negative correlation with HDL-C level ($p < 0.05$). The present study demonstrated increased mean concentration of Hcy in hs-CRP women with PCOS. **Conclusion.** Our results support the use of these biomarkers in the evaluation of potential risk for cardiovascular diseases and early prognosis and treatment implications.

Key words:
polycystic ovary syndrome; cardiovascular diseases; risk factors; homocysteine; c-reactive protein.

Apstrakt

Uvod/Cilj. Prevalencija metaboličkih poremećaja, gojaznosti i insulinske rezistencije kod žena sa sindromom policističnih jajnika (PCOS) prisutna je u ranim fazama života i postavlja ovu grupu u rizik za rani razvoj kardiovaskularnih bolesti. Hiperhomocisteinemija i povecana aktivnost C-reaktivnog protein (CRP) imaju uticaj na promociju ateroskleroze. Cilj ove studije je bio da se proceni da li su vrednosti visoko senzitivnog (hs-CRP) i homocisteina (Hcy) povišene kod PCOS i da se razjasne njihove moguće veze sa gojaznošću, insulinskom rezistencijom i metaboličkim promenama. **Metode.** Serumski nivoi hs-CRP i plazma nivoi Hcy su bili analizirani kod 73 žene sa PCOS i 43 zdrave žene, zajedno sa kliničkim, antropometrijskim i hormonskim parametrima. **Rezultati.** Srednje vrednosti indeksa telesne mase (BMI), obima struka, odnosa kukova i struka kao i koncentracija luteinizirajuceg hormona, testosterona, androstenediona, indeksa slobodnog androgena, insulina na gladno, homeostatskog modela za procenu insulinske rezistencije (HOMA-IR), i Hcy su bili značajno povećane kod žena sa PCOS u poređenju sa zdravim ženama istih godina. Ustanovljena je pozitivna korelacija između hs-CRP i BMI, holesterola, insulina, triglicerida ($p < 0,001$) i značajna negativna korelacija sa luteinirajućim hormonom (LH), vezujućim globulinom polnih hormona (SHGB), HOMA-IR, HDL-C ($p < 0,001$). Koncentracija Hcy imala je značajnu negativnu korelaciju sa HDL-C ($p < 0,05$). **Zaključak.** Istraživanjem je ustanovljena povećana srednja koncentracija Hcy i hs-CRP kod žena sa PCOS. Naši rezultati ukazuju na opravdanost upotrebe ovih biomarkera u proceni potencijalnog rizika za kardiovaskularne bolesti i rane prognoze i implikacija lečenja.

Ključne reči:
jajnik, policistični, sindrom; kardiovaskularne bolesti; faktori rizika; homocistein; c-reaktivni protein.

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Introduction

One of the most frequent condition frequently seen in women of reproductive age is the polycystic ovary syndrome (PCOS). This condition affects 12%–19% of the female population and this usually depends on ethnicity and criteria used for diagnosing PCOS [1, 2]. The diagnosis for PCOS is defined by the Rotterdam classification from 2003 where at least 2 out of 3 criteria must be present: oligo and/or anovulation, clinical and/or biochemical hyperandrogenism and polycystic ovaries on ultrasound [3]. This heterogeneous endocrine disorder is presented by different clinical sub-phenotypes and the phenotype prevalence generally depends whether it is diagnosed in the tertiary health care setting or detected randomly in the unselected population. This may be due to several reasons like race/ethnicity predominance, severity of clinical manifestation, access to medical care, degree of obesity and severity of hirsutism, which are the complaints most likely to affect quality of life and reason to seek medical assistance [4]. Apart from the well-known characteristics of polycystic ovary syndrome, these women are facing many other endocrine disturbances including fertility problems, insulin resistance (IR), impaired glucose tolerance, hyperinsulinemia, dyslipidemia and assemblage of metabolic syndrome profile. Data showed an increased diabetes risk, hypertension all of which are cardiovascular risk factors [5, 6]. These risks can be aggravated by the PCOS management strategies and by the presence of high body mass index (BMI). Some important therapies, like taking oral contraceptive pill (OCP) affecting the reproductive features such as menstrual cycle regulation and hirsutism management, may also increase cardiovascular risk [7, 8]. Obesity and excess weight are major chronic diseases in Western world countries. In general, the obesity and insulin resistance increase type 2 diabetes (T2DM) and cardiovascular disease (CVD). Similarly, in the PCOS obesity stimulates IR and promotes reproductive and metabolic disarrangements [9]. The metabolic features, combined with excess weight often seen in PCOS, make these young women a high-risk group. Chronic inflammation may be one of the factors contributing to obesity and obesity-related disorders, including atherosclerosis and endothelial dysfunction, diabetes, and steatosis [10]. These changes are present at the adipose tissue level but also liver, muscles and macrophages usually because of altered homeostasis of inflammatory cytokines. Adipose tissue, is a potent endocrine organ which synthetizes and releases cytokines, acute-phase proteins, and inflammatory mediators. These molecules have paracrine, autocrine or systemic function influencing glucose metabolism, energy balance, proinflammatory and anti-inflammatory activities [11]. Serum markers of low-grade chronic inflammation are being increasingly recognised as predictors of cardiovascular disease over the past years. Homocysteine (Hcy), a thiol-containing amino acid, is produced by the intracellular demethylation of methionine. Total Hcy (tHcy) represents the sum of all forms of Hcy including oxidised, protein-bound and free Hcy. Accumulation of Hcy is usually seen as a result of the defect in enzymatic pathway [12]. Several studies indicate that non-enzymatic factors also can influence Hcy levels including age, gender, nutrition and smoking [13]. Wald et al. [14] found a significant associations between Hcy concentration and the risk of ischemic heart disease and deep vein thrombosis. According to the authors, the results of the meta-analysis provide strong evidence for a causal relationship between elevated blood Hcy and cardiovascular disease. High sensitive C-reacitve protein (Hs-CRP) is an acute phase protein produced by the liver, directly secreted by adipose tissue, but also there are suggestions that it is produced in the atherosclerotic lesion, smooth muscle cells and macrophages [15].

There was an increased interest of use of Hcy and hs-CRP as early markers for early subclinical inflammation and atherosclerosis to improve a risk stratification in the asymptomatic individuals [13, 15].

The present study aimed to evaluate the relationships of plasma Hcy and hs-CRP levels with the anthropometric and biochemical parameters and their correlation with the metabolic profile of women with PCOS.

Methods

A cross-sectional study was conducted at the Department of Clinical Chemistry, University Clinic for Gynecology and Obstetrics, the Republic of North Macedonia. The study and all procedures were approved by the Ethics Committee for Research on People and Animals, at the Medical Faculty in Skopje, University St.” Cyril and North Methodius”, Macedonia. All participants in the study signed the informed consent before their participation in the study.

Seventy-three premenopausal, 18 to 40-year old women with POCS, based on the diagnostic criteria from the Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group, were involved in this study [3]. The control group consisted of 43-age-matched healthy females who volunteered for this study. All participants completed a questionnaire that included age, marital status, lifestyle habits and use of vitamin supplementation. The participants’ anthropometric assessment included the body weight, height, waist and hip circumference. The patients with abnormal levels of prolactin, thyroid hormones, renal or hepatic dysfunction, diabetes type 1 or 2 and congenital adrenal hyperplasia were excluded. The patients using any medication like hormonal supplementation and insulin sensitizers, or any vitamins in a period of 6 months were excluded.

Biochemical measurements

All blood samples were taken from the subjects after 12–14 hours overnight fasting between 3rd–7th day of the menstrual cycle, or at any given day for women with absent menstrual cycles in previous two or more months for the evaluation of hormonal parameters. The blood samples were withdrawn at 8–10 am from an antecubital vein after 5 min rest in the supine position, allowed clot for at least 30 minutes and then centrifuged. The resulting serum and plasma were aliquoted, frozen and maintained at -40˚C. Serum follicle stimulating hormone (FSH), luteinizing hormone (LH),

estradiol (E2), testosterone (T), androstenedione (A), dehydroepiandrostenedione-sulphate (DHEA-s), prolactin (PRL), Sex hormone binding globulin (SHBG) and the insulin levels were measured by the chemiluminescent immunometrical assay (Immulite 2000 HP, Diagnostics Products Corp). Plasma glucose was performed by the glucose oxidase method (Cobas Integra 400 plus analyser, Roche Diagnostic). For the homocysteine analysis, blood was collected in ethylenediaminetetracetic acid (EDTA) containing tubes. Hcy was measured on the Cobas Integra plus analyser, using enzyme-cycling method. Homocysteine enzymatic assay has higher specificity due to very low interference from cystathionine (an intermediate product in homocysteine metabolism). The principle of this method is a reduction of the oxidised form of homocysteine into free homocysteine. Using Hcy S-methyl transferase (HMT-use) and co-substrate, S-adenosyl methionine (SAM), free Hcy transforms to form methionine (Met) and S-adenosyl homocysteine (SAH). In the presence of S-adenosyl homocysteine- hydrolase, SAH is hydrolyzed into adenosine and Hcy. The created Hcy re-enters into reaction in which is transformed into methionine and SAH. In this cyclic transformation of Hcy, adenosine is accumulated and it is transformed into inosine and ammonia in the presence of adenosine hydrolase. The enzyme glutamate dehydrogenase (GLDH) catalyses the reaction of ammonia with 2-ketoglutarate and nicotinamide adenine dinucleotide (NADH) to form NAD+, which results in a decline of the absorbency at 340 nm. The concentration of Hcy present in the sample is directly proportional to the amount of NADH converted to NAD+. Quantitative determination of hs-CRP was determined using particle enhanced turbidimetric assay on the Cobas Integra 400 plus analyser. The measuring range of hs-CRP was 0.1–20 mg/L, with a lower detection limit of 0.1 mg/L. This method was standardised by the method comparison to the Tina-Quant CRP high sensitive assay. The height and weight measurements were taken twice and the mean of two measurements was used to calculate the BMI. The waist circumference (WC) was measured midway between the superior border of the iliac crest and the lowermost margin of the ribs, using the waistline measure employed with subjects standing without clothing covering the waist area. The hip circumference was measured at the point with the maximum circumference over the buttocks. The waist to hip ratio (WHR) was calculated as a ratio between the waist and hip circumference. The presence of insulin resistance was determined by basal insulin concentrations, fasting glucose concentrations and homeostasis model assessment HOMA-IR was calculated as fasting insulin (mIU/L) x fasting glucose (mmol/L) / 22,5 [16]. The free androgen index (FAI) was calculated by the standard formula: testosterone/SHBG x 100.

Statistical analysis

All statistical procedures were done using the SPSS 17 software for Windows. The Kolmogorov – Smirnov test was performed for the normality of distribution of all variables. The data were expressed as the mean ± standard deviation. The comparisons between the groups were performed using the independent *t*-test. The correlation analyses between Hcy and hs-CRP and other variables was obtained using the Spearman's rank coefficient. The differences between groups were considered to be statistically significant for the $p \leq 0.05$.

Results

From 116 women included into the study, 73 were with PCOS and 43 were healthy women. All participants were Caucasian, representative of nationalities that live at the territory of the Republic North Macedonia. The anthropometric and hormonal characteristics of women with PCOS and the control group were summarised in Table 1.

There was no statistical differences between the mean age of two groups: the women with PCOS were 23.9 ± 3.9 years of age and the women without PCOS were 24.67 ± 4.8 years of age. The PCOS patients showed the significantly higher BMI (27.6 ± 6.2 kg/m^2) vs. control (25.2 ± 6.0 cm, $p < 0.05$), WC (96.3 ± 14.8 cm) vs. control (87.6 ± 16.2 cm), ($p < 0.001$) and WHR (0.87 ± 0.07) vs. control (0.81 ± 0.05 cm), ($p < 0.001$). The concentrations of LH, LH/FSH ratio, total testosterone, DHEA-S, and FAI were significantly higher in the PCOS group ($p < 0.001$). The serum levels of FSH (5.61 ± 1.7 mIU/L vs. 6.3 ± 1.34 mIU/L), ($p < 0.01$), E2 (56.55 ± 22.7 pg/mL vs. 44.7 ± 10.76 pg/mL), ($p < 0.05$) and SHBG (34.2 ± 22.2 pg/mL vs. 52.35 ± 20.35 nmol/L), ($p < 0.001$) were significantly lower in the PCOS group. We observed no significant difference between patients for TSH and PRL concentration (Table 1).

The PCOS patients had significantly higher concentration of Hcy (11.98 ± 2.88 mmol/L) compared with the control group (8.5 ± 3.0 mmol/L), ($p < 0.001$), and hs-CRP (3.02 ± 4.7 mg/L) vs. (2.5 ± 4.1 mg/L), ($p < 0.05$) (Table 2).

The mean concentration of fasting glucose was slightly higher in the PCOS group (5.2 ± 0.4 mmol/L) vs. the control group (5.2 ± 0.4 mmol/L), ($p < 0.05$). The markers of insulin resistance, fasting insulin (15.35 ± 14.0 mIU/L vs. 6.7 ± 3.6 mIU/L), ($p < 0.001$) and HOMA- IR (7.37 ± 5.5 vs. 1.5 ± 0.89), ($p < 0.001$) were significantly higher in the PCOS group compared to the control group.

We observed changes in the lipid parameters between two studied groups. The PCOS group had a statistically significantly higher concentration of cholesterol (4.9 ± 1.0 mmol/L) vs. the controls (4.4 ± 0.7 mmol/L), ($p < 0.01$), LDL-C (3.0 ± 0.9 mmol/L) vs. control (2.63 ± 0.69 mmol/L), ($p < 0.01$), triglycerides (1.24 ± 0.78 mmol/L) vs. the controls (0.78 ± 0.23 mmol/L), ($p < 0.01$). A significantly lower HDL-C was found in the PCOS women (1.22 ± 0.34 mmol/L) vs. the controls (1.43 ± 0.28 mmol/L), ($p < 0.05$) (Table 2).

In Table 3, the correlation parameters between Hcy and hs-CRP with the selected parameters in the PCOS group are presented.

The Hcy values showed a statistically significant inverse correlation with HDL-C ($p < 0.05$). There was no significant correlation between Hcy and the parameters of PCOS such as testosterone, the LH/FSH ratio or the FAI.

Table 1

The anthropometric and hormonal characteristics of woman with polycystic ovary syndrome (PCOS) and the control group

Variables	PCOS group (n = 73) mean ± SD	Control group (n = 43) mean ± SD	*p*
Age (years)	23.9 ± 3.9	24.67 ± 4.8	0.11
BMI (kg/m$^{2)}$	27.6 ± 6.2	25.2 ± 6.0	0.03
WC (cm)	96.3 ± 14.8	87.6 ± 16.2	0.001
WHR	0.87 ± 0.07	0.81 ± 0.05	0.001
FSH (mIU/L)	5.61 ± 1.7	6.3 ± 1.34	0.01
LH (mIU/L)	9.49 ± 4.6	4.6 ± 3.2	0.001
LH / FSH	1.75 ± 0.9	0.73 ± 0.54	0.001
PRL (ng/mL)	11.5 ± 5.3	12.7 ± 5.6	n.s
E2 (pg/mL)	56.55 ± 22.7	44.7 ± 10.76	0.05
TSH (mIU/L)	2.3 ± 1.6	2.3 ± 0.74	n.s
DHEA-S (ng/mL)	3.83 ± 2.6	3.1 ± 4.2	0.001
Testosterone (nmol/L)	2.26 ± 0.88	0.98 ± 0.38	0.001
FAI	9.2 ± 7.3	2.27 ± 1.54	0.001
SHBG (nmol/L)	34.2 ± 22.2	52.35 ± 20.35	0.001

SD – standard devation; FSH – follicle-stimulating hormone; LH – luteinizing hormone; PRL – prolactin; E2 – estradiol, INS – insulin; TSH – thyroid-stimulating hormone; DHEA-S – dehydroepiandrostenedione-sulphate; FAI – free androgen index; SHGB – sex hormone binding globulin; HOMA-IR – homeostasis model assessment for insulin resistance.

Table 2

The metabolic characteristics of woman with polycystic ovary syndrome (PCOS) and the control group

Variables	PCOS group	Control group	*p*-value
Fasting glucose (mmol/L)	5.2 ± 0.4	5.2 ± 0.4	0.05
Fasting insulin (mIU/L)	15.35 ± 14.0	6.7 ± 3.6	< 0.001
HOMA-IR	7.37 ± 5.5	1.5 ± 0.89	< 0.001
Cholesterol (mmol/L)	4.9 ± 1.0	4.4 ± 0.7	0.012
Trigliceride (mmol/L)	1.24 ± 0.78	0.78 ± 0.23	< 0.01
HDL-C (mmol/L)	1.22 ± 0.34	1.43 ± 0.28	< 0.05
LDL-C (mmol/L)	3.0 ± 0.9	2.63 ± 0.69	0.01
Homocysteine (mmol/L)	11.98 ± 2.88	8.5 ± 3.0	< 0.001
hs-CRP (mg/L)	3.02 ± 4.7	2.5 ± 4.1	< 0.05

HOMA-IR – homeostatic assessment model for insulin resistance; HDL-C – high-denisity lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; hs-CRP – high sensitive C-reactive protein.

Table 3

The correlation coefficients (r) between homocysteine (Hcy), highsensitivity-C-reactive protein (hs-CRP) and other clinical parameters

Correlation coefficients	Homocysteine		hs- CRP	
	r	*p*	r	*p*
Age (years)	0.064	0.540	-0.052	0.683
BMI (kg/m^2)	-0.013	0.911	0.669	0.0001
WC (cm)	-0.003	0.980	0.576	0.0001
WHR	-0.108	0.380	0.209	0.098
FSH (mIU/L)	-0.021	0.865	-0.097	0.455
LH (mIU/L)	0.126	0.311	-0.369	0.003
LH/FSH ratio	0.094	0.448	-0.270	0.035
DHEA-S (μg/mL)	0.022	0.867	0.51	0.710
Testosterone (nmol/L)	-0.006	0.964	-0.083	0.516
FAI	-0.032	0.810	0.335	0.012
SHBG (nmol/L)	0.034	0.800	-0.464	0.001
Glucose (mmol/L)	0.085	0.475	0.025	0.842
Insulin (mIU/L) -	-0.290	0.811	0.534	0.001
HOMA-IR	-0.053	0.657	-0.318	0.010
Cholesterol (mmol/L)	0.046	0.705	0.242	0.054
Triglycerides(mmol/L)	0.090	0.461	0.370	0.002
HDL-C (mmol/L)	-0.278	0.034	-0.512	0.001
LDL –C (mmol/L)	-0.025	0.860	0.276	0.038

FSH – follicle-stimulating hormone; LH – luteinizing hormone; FAI – free androgen index; SHGB – sex hormone binding globulin; HOMA-IR – homeostasis model assessment for insulin resistance; BMI – body mass index; WC – waist circumference; WHR – waist to hip ratio; DHEA-S – dehydroepiandrostenedione-sulphate; HDL-C-high density lipoprotein cholesterol; LDL-C – low density lipoprotein cholesterol.

The serum hs-CRP values showed a significantly strong positive correlation with BMI, WC, insulin, and triglycerides ($p < 0.001$). A significant inverse correlation was observed among hs-CRP and LH, SHGB, HOMA-IR, HDL-C.

Discussion

Our study results demonstrated that the patients with PCOS had the increased inflammatory markers compared to the age and BMI-matched control group, indicating that the inflammation seen in PCOS might be related to the presence of disorder rather than obesity.

PCOS is a proinflammatory disorder and the increased levels of circulatory inflammatory markers such as tumor necrosis factor alpha (TNF-alpha), interleukin-6 (IL-6) have were found in several studies [10, 16]. The reasons of presence of low level chronic inflammation in PCOS has not been clarified yet, and it remains uncertain whether it is connected with PCOS itself, or it is associated with obesity.

It was shown that both obesity and inflammation contribute to the metabolic complications for women of reproductive age. The state of the cardiovascular system in women with PCOS might be due to different factors, including the insulin resistance, androgen status and BMI [6]. However, excess androgen should be the most important component of PCOS [17].

The women with polycystic ovarian syndrome face a variety of metabolic disorders, among which dysfunction of insulin activity has the most significant effect on the disorders presented later in life. Obesity, hyperinsulinemia, hyperandrogenaemia and insulin resistance are some of the metabolic disorders which shadow this syndrome and rise a question of possible increased cardiovascular risk among these women. High-sensitivity CRP is an acute phase protein that is mainly produced by the liver as a response to multiple reasons mainly to the increased levels of systemic inflammatory cytokines TNF-α and IL-6 [15]. Hs-CRP is considered to be a sensitive marker of low-grade inflammation, Met-Sy and as a predictor of future cardiovascular diseases (CVD) [18]. During the past years, conflicting results were published regarding the presence of endothelial dysfunction and the low-grade chronic inflammation in PCOS. A number of reports indicated that the levels of hs-CRP are significantly elevated in the women with PCOS pointing at the strong relationship between BMI and visceral fat as a reason for the elevated CRP levels [19]. Escobar-Morreale et al. [20] found that hs-CRP is the most reliable circulating marker of low-grade chronic inflammation in the women with PCOS and elevated levels of CRP are a reflection of presence of chronic inflammation in this condition. In his study, González [21] revealed that hs-CRP concentration was higher in obese women than in normal-weight women, regardless of PCOS. Thus, hs-CRP elevations attributed to PCOS were concealed by the presence of obesity and were below the range to predict a metabolic or cardiovascular risk. On contrary, some researchers believe that CRP elevation is associated with endocrine disorders [22]. Cho et al. [23] in their study showed that the mean concentration of CRP in the women with PCOS was higher than in healthy individuals, but inter-individual biological variations between the patients and healthy controls were similar. The inverse correlation between CRP concentration and insulin cannot solely explain insulin resistance, but it is more likely that CRP can be a marker of metabolic changes that contribute to the syndrome. In this study, we were challenged, for the first time to our knowledge, to evaluate hs-CRP in a sample of adolescent woman with PCOS living in North Macedonia. Presented data are in agreement with results demonstrated in previous studies that have found the higher hs-CRP serum concentration in the women with PCOS [19, 24]. We found a significant strong correlation between the hs-CRP concentration and BMI and WC. Kurt et al. [25] and Güdücü et al. [26] reported that both, obese and lean women with PCOS had increased CRP concentrations when compared with the BMI match control groups [25, 26]. These authors suggest that increased body weight and central fat are major basis of metabolic aberrations associated with CVD in PCOS while hs-CRP is a marker indicating existence of low-grade chronic inflammation and increased CVD risk. A significant correlation was stated previously between CRP and insulin resistance. We found a strong correlation of hs-CRP and fasting insulin concentrations and HOMA-IR. In the PCOS patients, correlation of hs-CRP with HOMA-IR was related to the presence of abdominal obesity, but independent of WHR. This finding suggests the increased risk of early atherosclerosis and cardiovascular events in the PCOS women, which at the same time is more pronounced and dependent on accumulated central fat deposit. In a study with a Croatian cohort of PCOS women, with a low prevalence of obesity, a significant association was found between SHBG and CRP, independent of insulin resistance, measured by HOMA-IR, supporting that low SHBG concentrations can be an independent marker of enhanced cardiovascular risk in the females with PCOS [27]. In our study, we observed a significant strong negative correlation between hs-CRP and SHGB and a weak positive correlation with FAI. We did not find any correlation with the androgen levels. However, the hs-CRP levels correlated with LH and LH/FSH ratio, triglycerides and LDL-C. This emphasises that obesity and metabolic alterations have influence in low-grade chronic inflammation and elevated CRP in the PCOS woman.

Moderately increased Hcy has a cytotoxic effect on vascular endothelium where oxidative stress leads to endothelial dysfunction causing vascular remodelling [28]. The elevated plasma concentrations of Hcy are an independent risk factor for early atherosclerosis and other vascular diseases [14]. Celik et al. [29] found higher levels of Hcy in the PCOS women. Contrary, Morgante et al. [30] did not find the significant elevation of Hcy concentration in PCOS woman. The present study is the first one in relevant literature to demonstrate increased Hcy in the cohort of PCOS women living in North Macedonia. The relationship between Hcy and PCOS may be explained by the presence of the increased low-grade chronic inflammation which is one of the main pathophysiological mechanisms in PCOS. Schachter et al. [31] indicate in their studies that insulin resistance is the major determinant of Hcy in the PCOS woman. In the present study, no association was found between Hcy, fasting insulin, IR, body mass, or other variables of PCOS. Supporting

the present study, Kilic-Okman et al. [32] indicated that the age, BMI and insulin resistance were not a predictor for Hcy levels. We did not observe any correlation between Hcy and BMI in the PCOS women. However, our study demonstrated a negative correlation between Hcy and serum HDL-C concentrations. The metabolite of Hcy combined with the lower HDL-C levels can be an initiation of development or disruption of endothelial cells and vascular remodelling. Homocysteine concentrations are dependent on few parameters, such as tobacco smoking and B-vitamins and folic acid intake [33]. The strong side of this study was that all involved subject were non-smokers, no vegetarians, and none of them was using any vitamin supplements, so the factor that can influence Hcy concentrations were eliminated.

Conclusion

The results of this study demonstrated presence of the low-grade chronic inflammation and increased inflammatory markers among the North Macedonian adolescent woman with PCOS compared to the corresponding BMI-matched control group. There is a growing data of evidence indicating disturbed Hcy metabolism as well as confirmation of the presence of chronic inflammation in the PCOS women, leading to the increased CVR risk. Accordingly, the presence of insulin resistance was shown, accessed through the indices of insulin action as it was basal insulin and HOMA-IR model. The markers of low-grade chronic inflammation, such as hs-CRP, were associated with BMI and accumulated central fat as well as with the presence of insulin resistance and disturbed lipid metabolism, while the elevation of Hcy was related with lipid metabolism. This finding further confirmed the existence of a proinflammatory state in a woman with PCOS. Nonetheless, these results suggest that screening for Hcy and CRP-Hs status may be beneficial and used for early identification CVD in PCOS in term of taking early preventive measures. Screening for hyperhomocysteinemia can be valuable before the use of oral contraceptive pills at a young age. Lifestyle changes with diet and exercise interventions particularly in overweight category can be advised in terms of limiting factors which are associated with increased cardiovascular risk, insulin resistance, hypertension, and dyslipidemia.

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Received on May 4, 2017.
Revised on October 4, 2017.
Accepted on October 9, 2017.
Online First November, 2017.

ORIGINAL ARTICLE

UDC: 616.348/.351-006-033.2:617.55
https://doi.org/10.2298/VSP170720151B

The effect of routine lymphadenectomy of the hepatic basin on the duration of the liver resection due to colorectal carcinoma metastases

Uticaj rutinske limfadenektomije hepatičnog sliva na trajanje resekcije jetre zbog metastaza kolorektalnog karcinoma

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Abstract

Background/Aim. Today, lymphatic metastases are only a relative contraindication for the surgical treatment of colorectal carcinoma (CRC). The aim of this study was to evaluate the effect of routine lymphadenectomy of the hepatic basin on the duration of the liver resection for CRC metachronous liver metastases. **Methods.** A total of 50 patients with CRC metachronous liver metastases underwent the liver resection with routine hepatic basin lymphadenectomy. **Results.** Larger volume of metastases (in mL), the number of affected segments, and the number of metastases as well the diameter of the largest lesion (in mm), determine the duration of the liver resection itself and the surgical procedure overall. The duration of lymphadenectomy was 25–55 min (32.2 min on average). **Conclusion.** Routine lymphadenectomy of the hepatic basin following the liver resection for CRC metachronous liver metastases, minimally prolongs the duration of the operation.

Key words:
colorectal neoplasms; liver; lymphatic metastasis; surgical procedures, operative; treatment outcome.

Apstrakt

Uvod/Cilj. Metastaze su u današnje vreme samo relativna kontraindikacija za hirurško lečenje kolorektalnog karcinoma (KRK). Cilj ove studije je bio da se utvrdi uticaj rutinske limfadenektomije hepatičnog sliva na trajanje resekcije jetre zbog metahronih jetrinih metastaza KRK. **Metode.** Ukupno 50 bolesnika sa metastazama jetre KRK podvrgnuto je resekciji jetre sa rutinskom limfadenektomijom hepatičnog sliva. **Rezultati.** Veći volumen metastaza jetre (u mL), broj zahvaćenih segmenata jetre, broj metastaza u jetri i veličina najveće metastaze u jetri (u mm) određivali su dužinu trajanja resekcije jetre i operacije u celini. Vreme trajanja limfadenektomije iznosilo je od 25 do 55 min (prosečno 32,2 min). **Zaključak.** Rutinska limfadenektomija hepatičnog sliva kod resekcije jetre zbog metahronih metastaza KRK neznatno produžava vreme trajanja operacije.

Ključne reči:
kolorektalne neoplazme; jetra; neoplazme, limfne metastaze; hirurgija, operativne procedure; lečenje, ishod.

Introduction

Malignant cells within the lymph nodes (LNs) of the hepatic basin, originate from the liver metastasis (remetastasis, metastasis from metastases, tertiary metastasis, lymphatic metastasis) [1, 2]. Remetastasis originate from lymphogenic dissemination of the metastatic liver disease [3].

In the past, positive LNs of the hepatic basin were a contraindication for liver resection of metastases of colorectal carcinoma (CRC). Today, lymphatic metastases are only a relative contraindication for the surgical treatment of CRC liver metastases [4].

Macroscopically enlarged LNs are detected radiologically (preoperatively, perioperatively), visually, or by palpation (intraoperatively). The macroscopic examination of enlarged LNs was confirmed of malignancy in 2%–10% of patients (larger, hard or soft lymph node, pink or white) while the revealed malignancy range of 14%–30% surgically

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treated patients [5–8]. However, only the histopathological examination can reliably confirm the presence of malignancy in the LNs by detecting the malignant cells [9].

Based on the Japanese classification of gastric cancer and the General Rules for surgical and pathological studies of cancer of the biliary tract, Kokudo et al. [4] classified the LNs of the hepatic basin into seven groups (Figure 1).

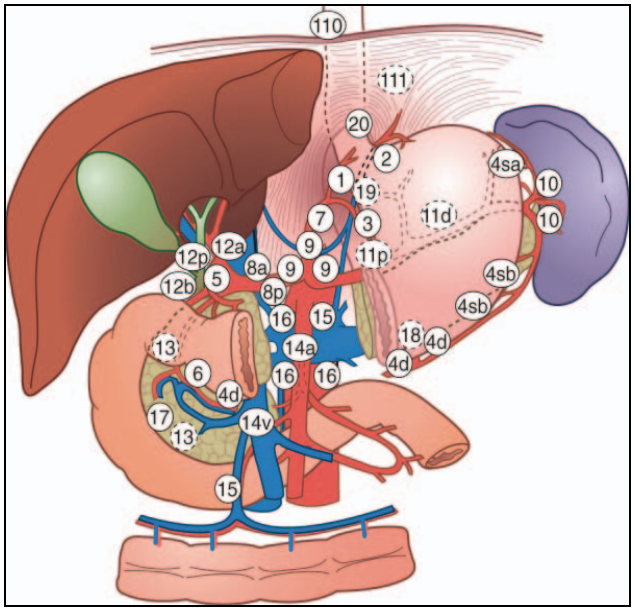

Fig.1 – Classification of lymph nodes (LNs) by group Gastric Cancer Association (JGCA) [4].
LNs of the hepatic basin: 8a – anterosuperior LNs along the common hepatic artery, 8p – posterior LNs along the common hepatic artery, 12a – LNs along the proper hepatic artery, 12p – LNs along the portal vein, 12h – LNs of the liver hilum, 12b – LNs along the bile duct, 13 – LNs posterior of the pancreatic head.

The location and number of the LNs involved is a significant prognostic factor of survival for the operated patients [4, 9–14].

Lymphadenectomy of the hepatic basin involves excision of the LNs around the hepatic pedicle – hepatoduodenal ligament (around the hilus of the liver, common bile duct, cystic duct, portal vein, hepatic artery), retropancreatic (posterior of the pancreatic head), and along the common hepatic artery (at its origin at the level of the celiac axis).

Lymphadenectomy can be standardized (around hepatic pedicle), or extensive [4, 10–12]. Standardized (localized) regional lymphadenectomy is limited lymphadenectomy in the area of the hepatic pedicle (hepatoduodenal ligament, retropancreatic, along the common hepatic artery) [4, 10–12]. Extended lymphadenectomy includes additional dissection of the LNs at the celiac axis (Group 9); however it does not affect survival significantly [4, 10–12]. The positive LNs at the celiac axis are a poor prognostic factor for survival, and there are no recommendations for extended lymphadenectomy [4, 10–12, 15].

Depending on the degree of dissection, lymphadenectomy can be routine, or based on the selection of the LNs. Routine lymphadenectomy involves the removal of all the LNs of the hepatic basin (pedicle), i.e., complete dissection along hepatoduodenal and hepatogastric ligaments. In contrast to routine lymphadenectomy, a sampling of selected LNs includes their removal from the hepatic basin after macroscopic validation, by palpation or by imaging methods [15, 16].

Selective lymphadenectomy is performed in strictly defined patients (young patients, with no comorbidities, or when chemotherapy options are exhausted or chemotherapy is not tolerated) [17].

There are no randomized trials of liver resection for CRC metastases, with and without lymphadenectomy [13, 18]. However, existing reports vary with regards to the recommended extent of lymphadenectomy and the observed effects [4, 6, 10, 12, 19].

The prognostic significance of lymphadenectomy is that it fascilitates early detection of recurrence [20, 21]. Detection of positive LNs leads to a timely administration of adjuvant chemotherapy, and it prolonges the survival [4, 18, 22, 23].

Methods

This prospective study included the patients with CRC liver metastases who underwent surgery for primary CRC as well as the metachronous liver metastasis. The study includes 50 patients operated during a period ranging from 01.01.2015 to 01.07.2016, at the First Surgical Clinic, the Clinical Center of Serbia in Belgrade.

The inclusion criteria for the patients in this study were: the computed tomography (CT) or magnetic resonance imaging (MRI) verification of surgically treatable metachronous liver metastasis; complete resection of all metastatic lesions regardless of their number, size, and location, and complete regional lymphadenectomy of the hepatic basin.

The exclusion criteria were: the presence of extrahepatic and distant metastasis; the patients who underwent surgery for another carcinoma; the patients diagnosed with other types of cancer; the patients requiring multiple or combined resections of other organs; macroscopically identifiable malignant lymphadenopathy of the hepatic basin, without suspicion or evidence of other malignant lymphadenopathies.

Routine lymphadenectomy was performed as the dissection of the hepatoduodenal ligament, along the common hepatic artery and retropancreatically (according to the method of Moszkowicz et al. [16]). The following groups of hepatic basin lymph nodes were removed by routine lymphadenectomy: 8a, 8b, 12a, 12p, 12b, 12h, 13.

Clinical parameters of the patients with CRC and liver metastases

The demographic factors and comorbidity based on disease history are presented in the Table 1.

The surgical parameters of the study were the number, size, and location of the liver metastases; the number of affected liver segments; the selection of the surgical procedure – the liver resection (anatomical and nonanatomical, major or minor); the number of drains; and the duration of the postoperative drainage (Table 2).

Table 1

The demographic factors and comorbidity

Parameter	Patients, n (%)
Sex	
men	35 (70.0)
women	15 (30.0)
Age (years)	
< 40	5 (10.0)
40–49	5 (10.0)
50–59	10 (20.0)
60–69	18 (36.0)
70–79	11 (22.0)
80–89	1 (2.0)
Comorbidity	
no	24 (48.0)
yes	26 (52.0)
ASA score	
1	10 (20.0)
2	28 (56.0)
3	12 (24.0)
Lymphadenopathy on CT/MRI	
no	43 (86.0)
yes	7 (14.0)
Volume of the liver (mL)	
1,000–1,499	28 (56.0)
1,500–1,999	15 (30.0)
2,000–2,499	7 (14.0)
Volume of liver metastases (mL)	
< 60	20 (40.0)
60–119	19 (38.0)
120–179	8 (16.0)
> 180	3 (6.0)
Chemotherapy before liver resection	
no	32 (64.0)
yes	18 (36.0)

ASA – American Society of Anaesthesiologists;
CT – computed tomography; MRI – magnetic resonance.

Results are presented using the descriptive statistics tools (measures of central tendency – an arithmetic mean; variability – the standard deviation; relative numbers) as well as the analytical statistical evaluation of the significance of the difference (parametric – *t* test; nonparametric - χ^2 test).

Results

The standard histopathological analysis detected 12% positive LNs of the hepatic basin (6 patients). The immunohistochemical analysis confirmed an additional 16% positive LNs (n = 8).

The postoperative complications following the liver resection with routine lymphadenectomy (nonspecific postoperative complications) were detected in 8 (16%) patients: the fluid collection at the site of liver resection – 3 (6%) patients, the abscess at the site of resection – 2 (4%) patients, the partial portal vein thrombosis, the biliary fistula and surgical wound infection each in one 1 (2%) patient.

The influence of selected parameters on the occurrence of postoperative complications after the liver resection following routine lymphodenectomy is shown in Table 3 and Table 4.

Table 2

The surgical parameters

Parameter	Patients, n (%)
Number of liver metastases	
1	14 (28.0)
2	23 (46.0)
3 or more	13 (26.0)
Largest metastasis diameter (mm)	
< 20 mm	7 (14.0)
20–50 mm	26 (42.0)
> 50 mm	17 (34.0)
Lobar localisation of metastasis	
left	5 (10.0)
right	20 (40.0)
both	25 (50.0)
Number of the affected segments	
1	7 (14.0)
2	18 (36.0)
3 or more	25 (50.0)
Type of liver resection	
nonanatomical	27 (54.0)
anatomical	23 (46.0)
Size of liver resection	
small	25 (50.0)
large	25 (50.0)
Number of drains	
1	2 (4.0)
2	38 (76.0)
3 or more	10 (20.0)
Duration of the postoperative drainage (days)	4 (8.0)
1–3	27 (54.0)
4–5	19 (38.0)
6 or more	

The features of postoperative complications following routine lymphadenectomy are shown in Table 5 and Table 6.

Routine lymphadenectomy of the hepatic basin performed in the Belgrade First Surgical Clinic is shown in the Figure 2. The duration of lymphadenectomy was 25–55 min (average 32.2 min).

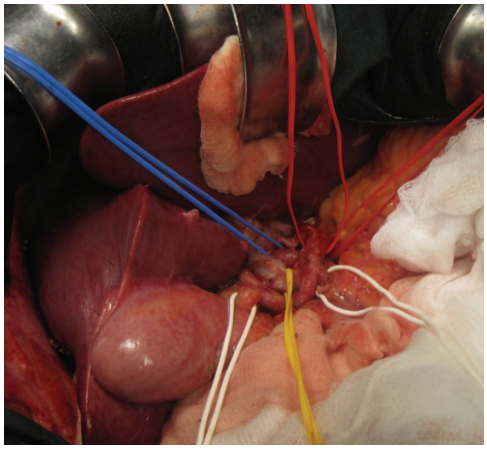

Fig. 2 – Routine lymphadenectomy of the hepatic basin (First Surgical Clinic, VII Department).

Table 3

The influence of selected parameters on the occurrence of postoperative complications after the liver resection following routine lymphadenectomy

Parameter	Postoperative complications		*p*-value (χ^2 test)
	No	Yes	
	n (%)	n (%)	
Sex			
men	27 (77.1)	8 (22.9)	0.086
women	15 (100.0)	0 (0.0)	
Chemotherapy before liver resection			
no	27 (84.4)	5 (15.6)	1.000
yes	15 (83.3)	3 (16.7)	
Comorbidity			
no	18 (75.0)	6 (25.0)	0.132
yes	24 (92.3)	2 (7.7)	
ASA score			
1	7 (70.0)	3 (30.0)	0.567
2	25 (89.3)	3 (10.7)	
3	10 (83.3)	2 (16.7)	
Lymphadenopathy seen on imaging (CT/MRI)			
no	37 (86.0)	6 (14.0)	0.580
yes	5 (71.4)	2 (28.6)	
Type of liver resection			
nonanatomical	24 (88.9)	3 (11.1)	0.444
anatomical	18 (78.3)	5 (21.7)	
Size of liver resection			
small	19 (76.0)	6 (24.0)	0.247
large	23 (92.0)	2 (8.0)	
Lobar localisation of metastases			
left	5 (100.0)	0 (0.0)	0.668
right	16 (80.0)	4 (20.0)	
both	21 (84.0)	4 (16.0)	

n – number of patients; CT – computed tomography; MRI – magnetic resonance imaging; ASA – American Society of Anesthesiologists

Table 4

The influence of selected parameters as a function of their mean value, on the occurrence of postoperative complications after the liver resection following routine lymphadenectomy

Parameter	Postoperative complications		*p* value
	n	mean ± SD	
Age			
no	42	60.64 ± 11.25	0.344[a]
yes	8	57.25 ± 10.25	
Chemotherapy before the liver resection			
no	15	5.20 ± 2.11	0.933[b]
yes	3	4.67 ± 1.15	
Volume of the liver (mL)			0.904[a]
no	42	1,542.90 ± 340.14	
yes	8	1,518.25 ± 325.07	
Volume of CRC metastases of (mL)			
no	42	71.69 ± 45.31	0.019[b]
yes	8	122.75 ± 57.63	
Number of drains			
no	42	2.14 ± .47	0.105[b]
yes	8	2.50 ± .93	
Duration of postoperative drainage (days)			
no	42	5.52 ± 2.76	0.015[b]
yes	8	11.25 ± 8.71	
Number of the affected segments			
no	42	2.79 ± 1.34	0.567[b]
yes	8	2.50 ± 0.93	
Number of hepatic metastases			
no	42	2.98 ± 2.05	0.759[b]
yes	8	2.63 ± 1.69	
Size of the largest metastasis (mm)			
no	42	39.40 ± 22.59	0.031[b]
yes	8	70.50 ± 38.81	

n – number of patients; SD – standard deviation; [a]*t*-test, [b]Mann-Whitney *U* test.

Table 5

The occurrence of postoperative complications as a function of the duration of routine lymphadenectomy and the liver resection

Postoperative complications	n	mean ± SD	*p* value (*t*-test)
Duration of routine lymphadenectomy (min)			
no	42	32.26 ± 7.00	0.890
yes	8	31.88 ± 3.72	
Duration of liver resection (min)			
no	42	123.10 ± 53.35	0.011
yes	8	175.00 ± 47.21	

n – number of patients; SD – standard deviation.

Table 6

The frequency and distribution of postoperative complications following routine lymphadenectomy

Authors	Year	Number of patients	Type of lymphadenectomy	Postoperative complications	Type of postoperative complication
Yuasa et al. [31]	1994	52	Extended	42%	Stenosis or occlusion of hepatic artery
Elias et al. [5]	1996	100	Extended	17%	Biliary fistula Pulmonary effusion
Kokudo et al. [4]	1999	160	Routine	/	Lymphorrhea, Stenosis of bile duct
Jaeck et al. [10]	2002	221	Routine	/	Lymphorrhea, Biliary complications
Ercolani et al. [33]	2004	120	Routine	0.83%	Bleeding
Moszkowicz et al. [16]	2012	76	Routine	/	Pancreatic fistula Lymphorrhea Bleeding, ischemia Biliary fistula

Discussion

Some authors have demonstrated a beneficial effect of routine lymphadenectomy added to the liver resection in prolonging the five-year survival over 30% [4, 9, 10, 18, 24–26].

Routine hilar lymphadenectomy was performed by 27% of the surgeons, with the same percentage of surgeons who made the sampling of selected lymph nodes [4].

Numerous studies indicate that the dissection of lymph nodes of the hepatic basin reveals a higher frequency of the micrometastasis than detectable macroscopically, or by palpation [4–6, 9, 12, 15, 16, 26–28].

Gallinger et al. [29] and Bradatsch et al. [30] argue that if lymphadenopathy is not confirmed by imaging methods (ultrasound, CT, MRI), it is not necessary to perform routine lymphadenectomy because it prolongs surgery and increases the incidence of postoperative complications [31], although the postoperative complications are rare and relatively mild [5, 10].

The postoperative complications, following the liver resection with routine lymphadenectomy include: stenosis of the hepatic artery, postoperative bleeding, lymphorrhea, biliary complications, pancreatic fistula [4, 5, 16, 31–33].

All postoperative complications in our study were nonspecific and managed conservatively (percutaneous drainage, antibiotic therapy, endoscopic retrograde cholengiopancreatography – ERCP), which is in accordance with the published results. The liver resection for CRC metastasis was prolonged by adding lymphadenectomy for 20 ± 12.5 min, without mortality in the study [23]. The authors suggest performing routine lymphadenectomy associated with the liver resection because there are no reliable methods for the confirmation of the presumed malignant lymphadenopathy within the hepatic basin based on CT imaging and intraoperative examination [23].

The surgical duration of lymphadenectomy in this study ranged from 25–55 min (average 32.2 min). The duration was gradually decreased over time as a result of improvement in surgical techniques.

Conclusion

The larger volume of liver metastases (mL), the number of the affected segments, the number of metastasis and the diameter of the largest lesion (mm) prolong the duration of liver resection itself and duration of the entire operation.

Routine lymphadenectomy of the hepatic basin, following the liver resection for CRC metastatses, minimally prolongs the duration of the operation.

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Received on July 20, 2017.
Revised on September 21, 2017.
Accepted on October 09, 2017.
Online First October, 2017.

ORIGINAL ARTICLE

UDC: 616-058:616.24
https://doi.org/10.2298/VSP161223147V

Predictors of quality of life of patients with chronic obstructive pulmonary disease

Prediktori kvaliteta života bolesnika sa hroničnom opstruktivnom bolesti pluća

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Abstract

Background/Aim. Chronic obstructive pulmonary disease (COPD) has a significant impact on quality of life of patients. We investigated which demographic and social characteristics can predict the global quality of life (QoL) of COPD patients. **Methods.** The patients (n = 288) were divided into three groups according to the stage of disease: Group I = stage 0 – at risk; Group II = Stages I and II; Group III = stages III and IV. The patients fulfilled a questionnaire related to the demographic and social characteristics and the validated multidimensional questionnaire – Serbian version of the St. George's Respiratory Questionnaire (SGRQ). The Student's *t* test, χ^2 test, ANOVA, univariate and multivariate logistic regression tests were used for statistical analyses. **Results.** In the group I, prevailed the men, employed persons, with a moderate financial status and no family history of COPD. In the group II dominated women, pensioners, with a moderate financial status, duration of illness up to five years, and no family history of COPD. In the group III prevailed women, unemployed persons, a moderate financial status, COPD duration up to 5 years and no family history of COPD. The predictors of the Symptoms score were grades of COPD and duration of the disease, and the predictors of Activity grades of COPD, sex, age and financial status. All variables were found to have a statistically significant relationship in the Impact score in the pre-analyses, were also significant in the univariate regression model. They were age, employement status, financial status and COPD duration. The same predictors that significantly contributed to the explanation of the Impact score, contributed to the explanation of the Total score on SGRQ. In the multivariate regression model, the predictors of the Activity score, Impacts score and Total score were the COPD grade and financial status; only the COPD grade contributed to the explanation of the Symptoms score. **Conclusion.** Financial status is the most important social factor, and the grade of COPD is the best disease-related predictor of QoL of COPD patients.

Key words:
pulmonary disease, chronic obstructive; quality of life; demography; socioeconomic factors; surveys and questionnaires; serbia.

Apstrakt

Uvod/Cilj. Hronična opstruktivna bolest pluća (HOBP) je progresivna i ireverzibilna bolest sa negativnim uticajem na kvalitet života obolelih. Cilj našeg ispitivanja bio je da odredi demografske i socijalne faktore obolelih koji su prediktori kvaliteta života. **Metode.** Bolesnici (n = 288) bili su podeljeni u tri grupe: I grupa – bolesnici u stadijumu 0 HOBP, u riziku; II grupa – bolesnici u stadijumu I i II HOBP; grupa III – bolesnici u stadijumu III i IV HOBP. Bolesnici su ispunjavali dva upitnika – jedan sa demografskim i socijalnim podacima, a drugi, validiranu srpsku verziju upitnika bolnice "Sveti Đorđe" o respiratornim teškoćama – *St. George's Respiratory Questionnaire for Chronic Obstructive Pulmonary Disease* (SGRQ-C). Podaci su obradjeni pomoću Student *t*-testa, χ^2 testa, ANOVA testa, kao i univariatnom i multivariatnom metodom logističke regresije. **Rezultati.** U grupi I dominirali su muškarci, zaposleni, osrednjeg materijalnog stanja, bez porodične anamneze HOBP. U grupi II je bilo više žena, penzionera, osrednjih prihoda, bolovanja do pet godina, bez HOBP u porodici. Žene, nezaposleni, osrednjih prihoda, trajanja HOBP do pet godina, bez HOBP u porodici,

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dominirali su u III grupi. Prediktori za skor simptoma bili su stadijum bolesti i dužina bolovanja, dok su u skoru aktivnosti prediktori bili stadijum bolesti, pol, starost i finansijska situacija. Sve statistički značajne varijable u skoru uticaja u pre-analizama bile su značajne u univarijantnom regresionom modelu, a to su bili stadijum bolesti, starost, zaposlenje, finansijska situacija i dužina bolovanja. Isti prediktori objašnjavali su i ukupni skor u SGRQ. U multivarijantnom regresionom modelu, prediktori skora aktivnosti, skora uticaja i ukupnog skora bili su stadijum bolesti i finansijska situacija; samo stadijum bolesti učestvovao je u objašnjenju skora simptoma. **Zaključak.** Finansijska situacija je najbolji socijalni prediktor, a stadijum bolesti najbolji od bolesti zavisan prediktor kvaliteta života obolelih od HOPB.

Ključne reči:
pluća, opstruktivne bolesti, hronične; kvalitet života; demografija; socijalno-ekonomski faktori; ankete i upitnici; srbija.

Introduction

The World Health Organization (WHO) recognizes that chronic obstructive pulmonary disease (COPD) is of a major public health importance, causing huge economic burden not only to developed countries but even more to low and middle income countries, and in particular, to the vulnerable population [1]. Since COPD is progressive disorder, it has a significant negative impact on quality of life (QoL) of patients. Today, QoL is very important outcome measure in any chronic disease including COPD. After the importance of QoL in COPD patients has been increasingly recognized, several research groups started to study QoL of these patients [2–4] in more detail.

It is now known that COPD affects QoL by causing numerous physical, functional, psychological and social stigmata [5]. In order to measure health-related QoL in this chronic disease, several instruments were developed [6] and compared [7]. The most commonly used is the St George's Respiratory Questionnaire (SGRQ) [8], which was translated and validated into several languages [9] including Serbian [10].

We investigated the COPD patients-generated data of their social structure and which of these have the greatest impact on their QoL.

Methods

Patients

This investigation was performed from July to December 2016. A total of 288 outpatients suffering from COPD in a stable phase of the disease COPD entered this ethically approved study (Decision of Ethics Committee of Primary Health Center "Zemun" N^{o} 03-887/2). The responders were all the patients of Primary Health center "Zemun". The eligible criteria included confirmed diagnosis of COPD according to the Global Initiative for Obstructive Lung Disease (GOLD) criteria [11, 12]. All participants signed the written informed consent. The patients younger than 18 years, those with bronchial asthma, lung cancer or any other respiratory disease that might induce chronic airflow limitation, were excluded. The patients were divided into three groups according to the severity of their disease: Group I – stage 0 (risk group); Group II included stages 1 (mild) and 2 (moderate); and Group III included stages III (severe) and IV (very severe) airflow limitation.

Method

The respondents were asked to fill out a questionnaire including demographic (sex, age) and social characteristics (employment, self-estimated financial status). The patients also fulfilled the validated multidimensional questionnaire – Serbian version of SGRQ [10], which is designed to measure and quantify health-related status in the patients with chronic airflow limitation. The first part of this questionnaire ("Symptoms") evaluates symptoms (frequency of cough, sputum production, wheeze, breathlessness and the duration and frequency of attacks of breathlessness or wheeze). The second part has two components: "Activity" and "Impacts". The "Activity" section addresses activities that cause breathlessness or are limited because of breathlessness. The "Impacts" section covers a range of factors including influence on employment, being in control of health, panic, stigmatization, the need for medication, side effects of prescribed therapies, expectations for health and disturbances of daily life.

Statistical analysis

All calculations were performed using the Statistical Package for the Social Sciences (SPSS) statistical package, version 21. The baseline quantitative characteristics of patients were expressed as mean, median (M), standard deviations (SD) and rank, while categorical variables were expressed as frequencies and percentages. Statistical significance of differences between the groups was determined using the Student's *t* test, ANOVA and χ^2 test for qualitative variables. The univariate logistic regression test was used for variables found significant in pre-analyses, and those that gave statistically significant contribution to the explanation of dependent variable were tested by the multivariate model. Therefore, both univariate and multivariate models were used for prediction.

All statistical tests were considered significant with probability of 0.05.

Results

A majority of patients were females (54.5%). The women also dominated in the group I and group II of patients. The median age for all patients was 62 years, raising steadily from the group I to the group III (48, 64 and 68, respectively). A half of the patients (50.0%) were retirees, while the employed and un-

employed contributed almost equally to the second part (23.6% and 26. 4%, respectively). More than one third (36.8%) of patients considered their financial conditions to be moderate. There were also the patients who thought that it was bad (14.9%) or very bad (24.3). Only one quarter (24.0%) of patients considered their financial status to be good or very good. The COPD duration was up to 4 years in a great majority of patients in stage 0, which is (47.4% of all patients), while the duration up to 10 years and more was observed as dominant in patients from the groups II and III. Family history of COPD was denied by 63.6% of all patients.

Statistically significant differences were found for all characteristics of patients in relation to the stage of the disease.

Taking all together, in the group I (COPD stage 0), men and employed persons dominated, being of moderate financial status and illness duration up to 5 years with no family history of COPD. In the group II (stage I & II) there were dominatly presented women, pensioners, of moderate financial status with the illness duration up to 5 years and no family history of COPD. In the group III (stage III & IV), prevailed women, unemployed persons, of moderate financial status with COPD duration up to 5 years and no family history of COPD (Table 1).

The average values that the responders achieved at the Symptoms score, Activity score, Impacts score and Total score obtained by using the SGRQ instrument, are shown in Figure 1. The highest value was calculated for the Symptoms score (57.18), followed by the Activity score and Impact score (56.06 and 40.26, respectively). The Total score, which measured global quality of life, was 47.86, thus indicating that our patients had moderate QoL (Figure 1). All scores were expressed on the scale ranging from 0–100 (0 = the best; 100 = the worst).

In relation to the severity of disease, the groups differed significantly ($p < 0.001$) regarding all four scores of the questionnaire. The responders in the stadium III and IV had the highest values of all scores (Symptoms score: 58.1 ± 4.0; Activity score: 74.2 ± 29.5; Impacts score: 55.1 ± 22.5; Total score: 61.4 ± 19.5). The women had higher Activity score than men (59.6 ± 31.2 versus 51.8 ± 32.4). The eldest category (aged 71–95 years) had the highest Activity, Impact and Total scores (67.1 ± 32.4, 48.6 ± 25 and 55.7 ± 21.6 respectively). This difference among the categories was statistically significant ($p < 0.001$). On the contrary, no significant difference among the different categories regarding Symptoms score was found.

The responders having different employment status differed in the Impacts score and Total score ($p < 0.05$). The retired people had the highest values in both dimensions (Impacts score: 43 ± 25.3; Total score: 50 ± 22.8). The responders of the worst financial status had the highest values of the Activity score (75.9 ± 30.70), Impacts score (58.8 ± 22.4) and Total score (63.9 ± 20.3), the differences in these scores were statistically significant ($p < 0.001$). The patients with the longest history of disease had the highest values of the Symptoms score: (58.8 ± 4.2), Impacts score (46.3 ± 27.1) and Total score (52.3 ± 24.2), and these differences were statistically significant ($p < 0.001$). There was no difference between the patients with and without family history of disease (Table 2).

Table 1

Demographics and social characteristics of chronic obstructive pulmonary disease (COPD) patients

Parameters	Stage 0 (n = 80)	Stage I & II (n = 131)	Stage III & IV (n = 77)	*p*	All (n = 288)
Sex, n (%)					
male	50 (62.5)	54 (41.2)	27 (35.1)	< 0.001[a]	131 (45.5)
female	30 (37.5)	77 (58.8)	50 (64.9)		157 (54.5)
Age, (years) median (range)	48 (19–82)	64 (21–90)	68 (35–95)	< 0.001[b]	62 (19–95)
Employment, n (%)					
yes	33 (41.2)	28 (21.4)	7 (9.1)	< 0.001[a]	68 (23.6)
no	32 (40.0)	32 (24.4)	12 (15.6)		76 (26.4)
pensioner	15 (18.8)	71 (54.2)	58 (75.3)		144 (50.0)
Financial status, n (%)					
bad	3 (3.8)	14 (10.7)	26 (33.8)		43 (14.9)
very bad	25 (31.2)	37 (28.2)	8 (10.4)	< 0.001[a]	70 (24.3)
moderate	29 (36.2)	47 (35.9)	30 (39.0)		106 (36.8)
good and very good	23 (28.8)	33 (25.2)	13 (6.9)		69 (24.0)
Duration of COPD n (%)					
up to 5 years	71 (89.9)	52 (40.0)	12 (15.8)	< 0.001[a]	135 (47.4)
5 to 10 years	6 (7.6)	38 (29.2)	27 (35.5)		71 (24.9)
more than 10 years	2 (2.5)	40 (30.8)	37 (48.7)		79 (27.7)
Family history of COPD, n (%)					
yes	35 (44.3)	46 (36.2)	21 (28.4)	< 0.001[a]	102 (36.4)
no	44 (55.7)	81 (63.8)	53 (71.6)		178 (63.6)

Note: Stage I&II = Stage I Forced expiratory volume in the first second (FEV1 > 80%) + Stage II (FEV1 50–80%); Stage III & IV = Stage III (FEV1 30–50%) + Stage IV (FEV1 < 30%); [a]χ^2 test; [b]ANOVA test.

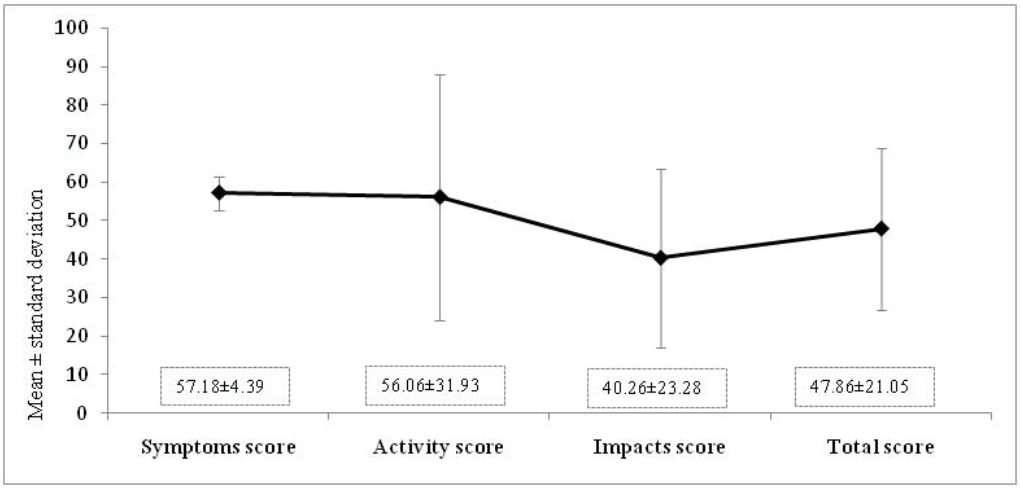

Fig. 1 – Average values obtained on four dimensions of St. George's Respiratory Questionnaire (SGRQ)

Table 2

Differences among the patients with various characteristics in dimensions of the St. George's Respiratory Questionnaire (SGRQ)

Parameters	Symptoms score mean ± SD	*p*	Activity score mean ± SD	*p*	Impacts score mean ± SD	*p*	Total score mean ± SD	*p*
Stage of COPD								
Stage 0	56.0 ± 4.6	<0.001[b]	42.5 ± 26.5	< 0.001[b]	28.8 ± 17.8	< 0.001[b]	37.5 ± 16.8	< 0.001[b]
Stage I & II	57.3 ± 4.3		53.7 ± 31.7		38.6 ± 22.2		46.3 ± 20.2	
Stage III & IV	58.1 ± 4.0		74.2 ± 29.5		55.1 ± 22.5		61.4 ± 19.5	
Sex								
male	56.9 ± 4.4	0.302[c]	51.8 ± 32.4	< 0.001[c]	38.0 ± 23.1	0.134[c]	45.3 ± 21.4	0.062[c]
female	57.4 ± 4.3		59.6 ± 31.2		42.1 ± 23.3		50.0 ± 20.6	
Age (years)								
19–30	57.1 ± 4.4		36.5 ± 22.4		27.9 ± 17.8		35.4 ± 15.6	
31–40	57.5 ± 4.3		58.5 ± 25.1		39.4 ± 20.3		48.2 ± 18.1	
41–50	56.7 ± 4.5	0.730[b]	55.2 ± 25	< 0.001[b]	39 ± 16.5	< 0.001[b]	46.9 ± 14.8	< 0.001[b]
51–60	56.8 ± 4.6		57.7 ± 32.3		39.7 ± 24		48 ± 21.7	
61–70	56.9 ± 4.5		50.5 ± 34.1		37.6 ± 23.8		44.7 ± 22.2	
71–95	57.8 ± 4.2		67.1 ± 32.4		48.6 ± 25		55.7 ± 21.6	
Employment								
yes	56.6±4.6	0.411[b]	48.3 ± 26.6	0.072[b]	33.1 ± 19.2	< 0.001[b]	41.6 ± 17.5	0.020[b]
no	57.6±4.2		58.7 ± 30.2		41.6 ± 21.6		49.4 ± 19.6	
pensioner	57.2±4.4		58.3 ± 34.6		43 ± 25.3		50 ± 22.8	
Financial status								
very bad	58.5±3.9		75.9 ± 30.7		58.8 ± 22.4		63.9 ± 20.3	
bad	57.1±4.4	0.163[b]	55.7 ± 32.4	< 0.001[b]	38.7 ± 21	< 0.001[b]	46.9 ± 19.9	< 0.001[b]
moderate	57.1±4.3		55.1 ± 31.3		41.1 ± 22.7		48 ± 20.3	
good and very good	56.6±4.6		45.4 ± 27.9		29 ± 19.6		38.6 ± 18.1	
Duration of COPD (years)								
up to 5	56.5±4.6	0.050[b]	51.4 ± 28.7	0.073[b]	34.8 ± 20.1	<0.001[b]	43.4±18.5	< 0.001[b]
5 to 10	57.8 ± 4.2		60.5 ± 32.1		44.2 ± 22.4		51.4±20.7	
More than 10	58.8 ± 4.2		59.9 ± 36.2		46.3 ± 27.1		52.3±24.2	
Family history of COPD								
yes	57 ± 4.5	0.721[c]	57.9 ± 28.6	0.495[c]	41.7 ± 21.7	0.425[c]	49.1 ± 18.7	0.433[c]
no	57.2 ± 4.4		55.2 ± 33.3		39.3 ± 23.8		47.1 ± 22	

COPD – chronic obstructive pulmonary disease; SD – standard deviation; [a]χ^2 test; [b]ANOVA test; [c]Student's *t*-test.

Table 3

Quality of life prediction for the patients with chronic obstructive pulmonary disease (COPD)

Dependent variables	Independent variables	Univariate linear regression analysis			Multivariate linear regression analysis		
		OR (95% CI)	*p*	Adjusted R square	OR (95% CI)	*p*	Adjusted R square
Symptoms score	Grade of COPD	0.17 (0.38–1.73)	< 0.001	0.02	0.13 (0.02–1.63)	0.045	0.02
	Duration of COPD	0.13 (0.08–1.29)	0.025	0.01	0.06 (0.39–1.01)	0.386	
Activity score	Grade of COPD	0.36 (11.12–20.47)	< 0.001	0.13	0.34 (9.49–20.56)	< 0.001	0.17
	Sex	0.12 (0.49–15.28)	0.037	0.01	0.09(-6.47–7.56)	0.878	
	Age	0.16 (0.10–0.60)	0.005	0.02	-0.02(-0.32-0.20)	0.657	
	Financial status	-0.26 (-12.07– -4.88)	< 0.001	0.06	-0.21(-10.24– -3.20)	< 0.001	
Impacts score	COPD grade	0.41 (9.78–16.44)	< 0.001	0.17	0.37 (7.58–16.16)	< 0.001	0.24
	Age	0.18 (0.11–0.46)	0.002	0.03	-0.03 (-0.23–0.22)	0.971	
	Employment	0.16 (1.30–7.84)	0.006	0.02	-0.04 (-5.28–2.71)	0.527	
	Financial status	-0.34 (-10.52–5.41)	< 0.001	0.11	-0.28 (-9.19– -4.21)	< 0.001	
	Duration of COPD	0.21 (2.83–9.14)	< 0.001	0.04	0.06 (-3.20–3.51)	0.928	
Total score	Grade of COPD	0.41 (8.91–14.93)	< 0.001	0.17	0.40 (7.67–15.41)	< 0.001	0.23
	Age	0.18 (0.10–0.42)	0.013	0.03	0.05 (-0.20–0.21)	0.950	
	Employment	0.14 (0.84–6.77)	0.012	0.01	-0.05 (-5.13–2.09)	0.409	
	Financial status	-0.32 (-9.21– -4.56)	< 0.001	0.1	-0.27 (-8.01– -3.51)	< 0.001	
	Duration of COPD	0.18 (1.82–7.56)	0.001	0.03	-0.03 (-3.79–2.26)	0.622	

OR – odds ratio; CI – confidence interval.

After testing of differences, the variables found to be statistically significant were tested by univariate regression model, and those that were significant were tested by the multivariate regression model. According to the univariate linear regression model, statistically significant predictors of the Symptoms score were: grade of COPD [odds ratio (OR): 0.17 (0.38–1.73); $p < 0.001$] and duration of the disease [OR: 0.13 (0.08–1.29), $p = 0.025$].

Statistically significant predictors of the Activity score in univariate analysis were: grade of COPD [OR: 0.36 (11.12–20.47); $p < 0.001$], gender [OR: 0.12 (0.49–15.28); $p = 0.037$], age [OR: 0.16 (0.10–0.60); $p = 0.005$], and financial status [OR: -0.26 (-12.07– -4.88), $p < 0.001$].

All variables found to have statistically significant relationship in the Impacts score in pre-analyses were also significant in the univariate regression model. The Impaets score was explained by the following variables: COPD grade [OR: 0.41 (9.78–16.44); $p < 0.001$], age [OR: 0.18 (0.11–0.46) $p = 0.002$], employement status [OR: 0.16 (1.30–7.84); $p = 0.006$], financial status [OR: -0.34 (-10.52–5.41); $p < 0.001$] and COPD duration [OR: 0.21 (2.83–9.14); $p < 0.001$].

The same predictors that significantly contributed to the explanation of the Impact score, contributed to the explanation of the Total score on SGRQ. These were independent variables: COPD grade [OR: 0.41 (8.91–14.93); $p < 0.001$], age [OR: 0.18 (0.10–0.42); $p = 0.013$], employement status [OR: 0.14 (0.84–6.77); $p = 0.012$], financial status [OR: -0.32 (-9.21–-4.56); $p < 0.001$) and COPD duration [OR: 0.18 (1.82–7.56); $p = 0.001$).

In the multivariate regression model, the predictors of the Activity score, Impacts score and Total score were COPD grade and financial status; these predictors explained 17% of the variance of dependent variable Activity score, 24% of the variance of Impacts score and 23% of the variance of the Total score. In the multivariate regression model, only the COPD grade contributed to the explanation of the Symptoms score (Table 3).

Discussion

Current approach to the investigation of QoL comprises the combination of objective indicators on different life domains with the subjective evaluation given by the individuals, using data on subjective well-being [13]. The subjective evaluation can be the limitation of this approach although such an approach is also recommended for investigations of QoL of patients suffering from COPD [14, 15]. According to these recommendations, we used this approach in our investigation of social factors that can predict QoL of COPD patients.

Several multi-country surveys presented at the 2011's European Respiratory Society's Annual Congress [16], revealed that COPD had the harmful impact on many aspects of quality of life. Our results showed that the financial factor as a social factor emerged in three dimensions that measured quality of life of the COPD patients, while the grade of COPD emerged as a statistically significant predictor of all four dimensions of this questionanaire.

Similarly to our results, it was reported that the decrease of financial status was one of the main reasons for patients' feelings of being unable to fulfill their life goals [17, 18]. It was reported that the lower social class in terms of the financial status had lower levels of QoL [17, 19–21]. A high percentage (39.2%) of our patients described their financial status as bad (14.9%) or very bad (24.3%), and therefore the poverty marked their financial status. Bad financial situation is probably influenced by their employment status since a great majority of our patients were unemployed or retired.

The disease severity (advanced stage of the disease) was also found to have a negative predictive effect on QoL of COPD patients [22]. In our study, the stage of COPD was in a positive correlation with the patients' age – our oldest patients had the most advanced stages of disease. It was shown that older age can also be a predictor of lower QoL of COPD patients [23, 24], although severe COPD may affect negatively QoL of even younger people [2, 7]. In very old people, any chronic disease is the main cause of lower QoL; the gender differences were insignificant [25].

However, it should be noted that the experience of aging may be influenced by some social and cultural factors that characterize different nations [26].

As far as gender is concerned, in our study, the women dominated in medium and advanced stages of disease, and their activity score was higher than that of the men. Several studies revealed that the female gender had poorer QoL than the male [27], especially in relation with a psychological well-being [28]. This was true even if women were younger and in the earlier stages of disease [29].

We conducted this study according to the recommendation that, in order to gain insight into QoL of COPD patients, both demographic and disease-specific impact and general impact of the disease should be used [30]. We found that the most important social factor that predict the QoL of our patients best was their financial status. The other disease-related predictor was the grade of COPD. The knowledge of not only the demographic but also the social characteristics of patient migh help the carers to predict quality of life of their patients. Better QoL of patients could be achieved by higher levels of positive social support, which perhaps may be influenced by efforts of health care providers in this sense. Since the main goal of medical care is to improve and maintain QoL of the patients [31, 32], we believe that our results might contribute to this ultimate goal achievement.

Conclusion

Financial status is the most important social factor that can best predict quality of life (QoL), and the grade of COPD is the best disease-related predictor of QoL of COPD patients.

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Received on December 23, 2016.
Revised on September 8, 2017.
Accepted on October 10, 2017.
Online First October, 2017.

ORIGINAL ARTICLE

UDC: 616-002:616.61-008.6-08
https://doi.org/10.2298/VSP160912155D

Serum C-reactive protein and nutritional parameters in hemodialysis patients

Serumski C-reaktivni protein i nutritivni parametri kod bolesnika na hemodijalizi

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Abstract

Background/Aim. Inflammation is the most important factor in the genesis of vascular complication in the end-stage renal disease. The serum C-reactive protein (CRP) level is a sensitive marker of systemic inflammation as well as a predisposing factor for cardiovascular and all cause mortality in patients on hemodialysis. Albumin is the negative acute phase protein and its synthesis declines during the inflammation. The patients undergoing hemodialysis have a high prevalence of protein-energy malnutrition, due to reduced protein synthesis and increased degradation. The low serum albumin levels in these patients originate from the complex setting of conditions with systemic inflammatory response as a major cause, malnutrition and overhydratation. The aim of this study was to determine the prevalence of elevated CRP levels in the dialysis patients and to analyse its correlation with serum albumin levels and other parameters of nutritional status. **Methods.** The study included 49 patients on maintenance hemodialysis at the Department of Hemodialysis, Clinic of Nephrology, Military Medical Academy, Belgrade, Serbia. In order to analyse the parameters, the blood samples were taken during the arteriovenous fistula (AVF) puncture and before the second weekly dialyse. The following parameters were determined: serum levels of urea and creatinine before and after the dialysis procedure, CRP, hemoglobin, fasting glycemia, total cholesterol, triglycerides, albumins, iron, glycosylated hemoglobin (HbA1c), fasting insulinemia and C-peptide only before the dialysis. **Results.** Out of 49 patients on maintenance hemodialysis, 37 (75.5%) were males and 12 (24.5%) females with the average age of 56.04 ± 13.93 years. The average duration of the dialysis treatment was 7.37 ± 5 years. The high serum CRP levels (more than 3 mg/L) was found in 65.3% of patients. Singificantly more diabetic patients were observed in the group with the higher CRP levels (n = 12) compared to the group with the normal CRP levels (n = 3) ($p \leq 0.05$). A significant positive correlation was found between the CRP value and urea values after the dialysis procedure. We found negative correlation between the CRP values and serum albumin, HbA1c, total cholesterol and triglyceride levels, with no statistical significance. **Conclusion.** Our study observed a high rate of inflammation in the dialysis patients presenting as high frequency of the elevated CRP levels in the examined group. Negative correlation between CRP levels and serum albumin as well as with some other parameters of nutritional status, suggests that chronic inflammation may be the missing link that actually connect protein energy malnutrition with high morbiditiy and mortality rate in these patients.

Key words:
albumins; c-reactive protein; inflammation; nutritional status; renal dialysis.

Apstrakt

Uvod/Cilj. Inflamacija je jedan od glavnih faktora odgovornih za nastanak vaskularnih komplikacija kod bolesnika sa terminalnom bubrežnom insuficijencijom. C reaktivni protein (CRP) se smatra senzitivnim markerom sistemske inflamacije, kao i faktorom koji doprinosi povećanom riziku od opšteg i kardiovaskularnog mortaliteta. Albumin je negativni protein akutne faze zapaljenja i njegova sinteza opada sa napredovanjem sistemske inflamacije. Za bolesnike na hemodijalizi poznato je da pate od visokog stepena proteinske malnutricije usled smanjene sinteze proteina i njihove pojačane razgradnje. Nizak nivo se-

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rumskog albumina kod tih bolesnika je posledica kompleksnog stanja koje podrazumeva sistemsku inflamaciju, malnutriciju i prekomernu hidrataciju. Cilj naše studije bio je da se ispita učestalost povišenog serumskog CRP-a u našoj populaciji dijaliznih bolesnika i da se utvrdi stepen njegove korelacije sa serumskim albuminom i drugim parametrima nutritivnog statusa. **Metode.** Ispitivanjem je obuhvaćeno 49 bolesnika na hroničnom programu hemodijalize u Centru za hemodijalizu Klinike za nefrologiju Vojnomedicinske akademije u Beogradu. Uzorci krvi za analizu uzimani su tokom punkcije arteriovenske fistule, a pre druge nedeljne hemodijalize. Određivanu su sledeći parametri: serumski nivo uree i kreatinina pre i posle procedure, CRP, hemoglobin, glikemija, ukupni holesterol, trigliceridi, glikozilirani hemoglobin (HbA1c), insulinemija i C-peptid pre hemodijalize. **Rezultati.** Od ukupno 49 dijaliznih bolesnika, bilo je 37 (75,5%) muškaraca i 12 (24,5%) žena, prosečne starosti 56,04 ± 13,93 godine. Dužina lečenja hemodijalizom je prosečno iznosila 7,37 ± 5 godina. Povišene vrednosti serumskog CRP-a (više od 3 mg/L) imalo je 65,3% bolesnika. U grupi bolesnika sa povišenim nivoom CRP-a u serumu, bilo je značajno više dijabetičara (n=12) u odnosu na grupu sa normalnim nivoom CRP-a u serumu (n = 3) ($p \leq 0,05$). Uočili smo postojanje značajne pozitivne korelacije između serumskog CRP-a i serumske uree nakon dijaliznog procesa. U ispitivanoj grupi postojala je negativna korelacija između serumskog CRP-a i serumskog albumina, HbA1c, ukupnog holesterola i triglicerida. Ta korelacija nije bila statistički značajna. **Zaključak.** Naša studija je potvrdila visok stepen sistemske inflamacije kod dijaliznih bolesnika izražene kroz visoku učestalost povišenih vrednosti serumskog CRP-a. Negativna korelacija između nivoa serumskog CRP-a i serumskog albumina kao i drugih nutritivnih parametara, sugeriše da hronična inflamacija može biti ključna karika koja povezuje proteinsku malnutriciju sa visokim morbiditetom i mortalitetom ovih bolesnika.

Ključne reči:
albumini; c-reaktivni protein; zapaljenje; nutritivni status; hemodijaliza.

Introduction

The patients undergoing hemodyalisis have a high prevalence of protein-energy malnutrition and inflammation, which is considered as the most important factor that generate several complications in uremic state. For a long time, C-reactive protein (CRP) has been presented as a marker of inflammation and advanced atherosclerosis. Some studies have shown that CRP may be a direct marker of vascular disease [1]. Serum levels of CRP are one to five times more prevalent in the dialysis patients than in the general population. On the other hand, it was observed that the level of inflammation shown by CRP, is the strongest predictor of serum albumin level [2]. Increased oxidative stress in malnutrition, combined with a chronic inflammation can lead to an increased risk of atherosclerotic lesions. Synthesis of albumin and other nutritional markers, such as prealbumin or transferrin, decreases with the duration of the inflammation as well as their serum levels. This shows that the higher values of the parameters of inflammation are associated with a poor nutritional outcome in the uremic patients. Accordingly, a new clinical significance of CRP is that it represents the index that reflects the general health situation of patients on dialysis [3].

Among protein malnutrition, uremic anorexia and chronic inflammation, there is an overlap which means that the conditions leading to malnutrition can actually cause the inflammation. Besides, a strong correlation between these occurances can explain the high cardiovascular (CVS) morbidity and mortality rate in these patients, despite improvemenet in dialysis technologies [4].

The aim of this study was to determine the prevalence of elevated CRP levels in the dialysis patients and to analyse its correlation with the serum albumin levels and other parameters of nutritional status.

Methods

The study included 49 patients on maintenance hemodialysis at the Department of Hemodialysis, Clinic of Nephrology, Military Medical Academy, Belgrade, Serbia. All patients were dialysed three times per week for 4 hours, using an arteriovenous fistula (AVF) as permanent vascular access. All participants signed the written informed consent to participate in this clinical research. In order to analyse the parameters, blood samples were taken during the AVF puncture and before the second weekly dialyse. The following parameters were determined: blood urea nitrogen (BUN) and creatinine before and after the dialysis procedure, CRP, hemoglobin, glycemia, cholesterol, triglycerides, albumins, iron, glycosylated hemoglobin (HbA1c), insulinemia and C-peptide only before the dialysis. The concentrations of biochemical blood parameters were obtained spectrophotometrically using the Simens Dimension Rxl Max analyzer. The value of CRP was calculated by the enhanced turbidimetric-immunoassay (PETIA) using the Simens Dimension Rxl Max analyzer, while C-peptide and insulin were determined by the CMIA (Chemiluminescent Microparticle Immunoassay) method using the Beckman Unicel DXI 800 machine.

The Kt/V value was calculated using the following formula by Daugirdas and Blake [5]:

$$Kt/V = -\ln(R-0.008xt) + (4-3.5 \times R) \times UF/W$$

R-Ratio = Post BUN/Pre BUN; t = time; UF/W = Ultrafiltrate Volume/Weight

K – urea clearance; t – dialysis time; V – urea volume distribution.

Statistical analysis

Complete statistical analysis of data was done by the statistical software package, SPSS Statistics 18. Most of the variables were presented as frequency of certain categories, while a statistical significance of differences was tested with the χ^2 test. In case of continuous data, the variables were presented as the mean value ± standard deviation (SD), median, minimal and maximal values. The Kolmogorov-Smirnov test was used for the evaluation of distribu-

tion of data. The Pearsons correlation analyses was used to establish the relation of parameters. All the analyses were estimated at $p < 0.05$ level of statistical significance.

Results

Out of 49 patients on maintenance hemodialysis, 37 (75.5%) were males and 12 (24.5%) females, with the average age of 56.04 ± 13.93 years. The average duration of the dialysis treatment was 7.37 ± 5 years. The baseline characteristics of our patients are shown in Table 1.

Table 1

Baseline clinical characteristics and C-reactive protein (CRP) values in 49 dialysis patients

Patients characteristics	Values
Sex, men/women, n (%)	37/12 (75.5/24.5)
Age (years), mean ± SD	56.04 ± 13.9
HD duration (years), mean ± SD	7.37 ± 5
DP/non DP, n (%)	15/24 (30.6/69.4)
CRP ≤ 3 mg/L, n (%)	17/32 (34.7/65.3)

HD – hemodialysis; DP – diabetic patients; non DP – nondiabetic patients; SD – standard deviation.

Of the total number of patients, 15 (30.6%) had diabetes mellitus, 4 patients had type 1 and 11 type 2 diabetes. Among all patients with diabetes, diabetic nephropathy was the initiator for a chronic renal failure stage 5 and for the beginning of dialysis treatment. The patients were divided into two groups according to the CRP values. The first group consisted of 17 (34.7%) patients with the normal CRP values (CRP ≤ 3 mg/L), while the second group consisted of 32 (65.3%) subjects with the increased CRP values (CRP> 3 mg/L). Singificantly more diabetic patients were observed in the group with the higher CRP levels (n = 12) compared to the group with the normal CRP levels (n = 3) ($p \leq 0.05$). The mean serum albumin level in the patients with high CRP (> 3 mg/L) was only numerically lower than in the patients with the low CRP values (37.06 ± 3.57 *vs* 37.65 ± 2.57 mg/L; $p \geq 0.05$). The correlation of CRP values with gender, age, smoking status, length of dialysis, diabetes mellitus and Kt/V are shown in Table 2.

During the comparison of the analyzed blood biochemical parameters, a significant positive correlation was found between the serum CRP value and urea values after the dialysis procedure (Figure 1). We found negative correlation between CRP values and serum albumin, HbA1c, total cholesterol and triglyceride levels. These correlations did not reach statistical significance (Table 3).

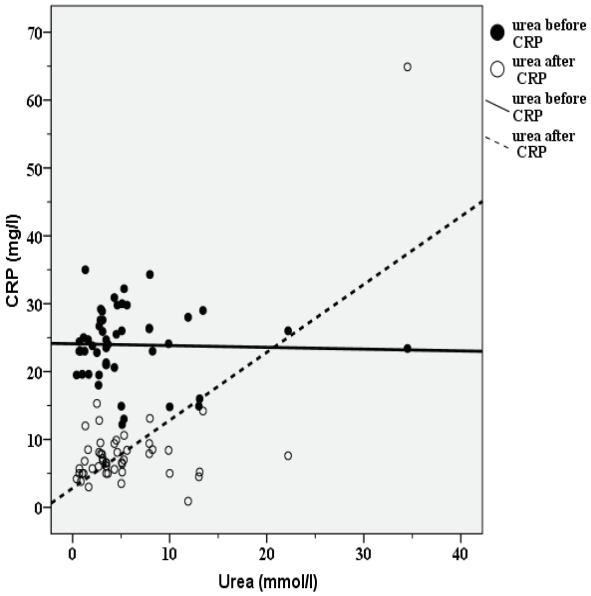

Fig. 1 – A significant positive correlation between the serum C-reactive protein (CRP) values and urea values after the dialysis procedure.

Table 2

Correlation between C-reactive protein (CRP) values and the different clinical characteristics of dialysis patients

Groups	Age	Gender	Smoke	HD durat.	DM	Kt/V
CRP (≤ 3 mg/L) (n = 17)						
r	-0.194	-0.577	0.223	0.104	0.064	-0.235
p	0.455	0.015	0.389	0.691	0.808	0.363
CRP (> 3 mg/L) (n = 32)						
r	0.202	0.232	-0.125	0.097	0.118	-0.209
p	0.268	0.200	0.497	0.597	0.521	0.251

HD – hemodialysis; DM – diabetes mellitus.

Table 3

Correlation between serum C-reactive protein (CRP) values with the differnet biochemical parameters of dialysis patients

Groups	BG	HbA1C	Insul.	CP	Urea		Creatinine		Alb	TChol	Tg
					before	after	before	after			
CRP (≤ 3 mg/L)											
r	0.278	0.035	-0.349	0.261	0.133	0.560	0.406	0.610	-0.312	-0.480	-0.451
p	0.281	0.893	0.170	0.312	0.610	0.019	0.106	0.009	0.223	0.051	0.069
CRP (> 3 mg/L)											
r	-0.009	-0.091	-0.087	0.244	-0.059	0.742	-0.255	0.058	-0.271	0.082	-0.122
p	0.959	0.619	0.640	0.195	0.749	0.001	0.158	0.754	0.134	0.654	0.506

BG – blood glucose; HbA1c – glycosilated hemoglobin; Insul – insulinemia; CP – C-peptide; Alb – albumin; TChol – total cholesterol; Tg – triglyceride.

Discussion

Inflammation is the most important factor in the genesis of vascular complication in the end stage renal disease (ESRD). The inflammation is potentially caused by decreased elimination of cytokines such as interleukin (IL)-1, tumor necrosis factor (TNF) and IL-6, or by accumulation of advanced glycation end products (AGEs). Metabolic acidosis is another potential factor that originated from a significant number of endocrine, musculosceletal and other metabolic abnormalities, leading to the enhanced inflammation. Oxidative stress and acompanied infection from several sites including graft or fistula, infections from bioincompatible dialysis membrane; periodontal infections or endotoxine exposure could be another cause that provoke an inflammatory reaction [6].

The serum CRP level is a sensitive marker of systemic inflammation as well as a predisposing factor of cardiovascular and atherosclerotic diasease [7]. CRP levels are 8-fold higher in the hemodialaysis patients than in the healthy controls, being a powerful predictor of all-cause and CVS death, even after a follow- up period of 4 years [8]. CRP is present in almost all atherosclerotic plaques, binds to modified low density lipoproteins (LDL) and activates the complement pathway. It was shown that human CRP can contribute to ishemic tissue damage of heart and brain in experimental rat models [6]. Many studies showed that the elevated CRP levels predict all cause and cardiovascular mortality in the patients on hemodyalisis. The 5-year survival rate and the risk of death was significantly poorer in Japanese population of chronic dialysis patients with the higher CRP levels [9]. In the study of Krane et al. [10], the CVS outcome in the dilaysis patients was influenced by CRP levels more than by LDL cholesterol levels.

The frequency of elevated serum CRP varies among different studies, from 20% do 65% of dialysis and predialysis patients [9–13]. In our study group, 65.3% of uremic patients had the serum levels of CRP higher than 3 mg/L. Abraham et al. [13] found the elevated CRP levels in 67% of dilaysis patients, while Iseki et al. [9] found the elevated CRP levels in only 21.5% of dialysis patients. These variations among studies may be caused by different biochemical techinques used to measure CRP levels. Observed differences could be also explained by the absence of consensus in related literature, regarding the optimal cutoff value of serum CRP levels to define the presence of inflammation in ESRD.

In addition, the level of the serum CRP increases with declining the serum albumin concentrations. Albumin is the negative acute phase protein and its synthesis declined during the inflammmation. The patients undergoing hemodyalisis have a high prevalence of protein-energy malnutrition due to the reduced protein synthesis and inreased degradation. The patients with end-stage of renal disease (ESRD) develop the low serum albumin levels due to the complex setting of conditions with the systemic inflammatory response as a major cause; malnutrition and overhydratation could also play an important role. It has been obeserved that the serum CRP levels are in correlation with anorexia [4]. In our study group, the serum albumin levels among dialysis patients with the low serum CRP values were higher than in those with the higher CRP levels. We found negative corellation between the serum CRP levels and the serum albumin, cholesterol and triglyceride levels; still, those differences and correlations did not reach statistical significance. Abraham et al. [13] found a significant negative correlation the between serum albumin and CRP levels among the dialysis population. The same results were obsereved in the diabetic patients with ESRD undergoing hemodyalisis [14]. A serum albumin level is one of the most important markers of malnutrition in the patients with ESRD, even when only slightly less than 4.0 g/dL. When the inflammatory process increases, there is a decrease in the serum albumin due its loss into extravascular space because of increased vascular permeability, or by increased consumption by cells locally, while decreased synthesis is a result of a direct cytokine inhibition [15]. High levels of CRP and low albumin levels often occur simultaneosly in the hemodialysis patients and are reffered together as a malnutrition-inflammation-atherosclerosis syndrome to emphasize its improtant interreaction leading to the advanced atherosclerotic cardiovascular disease (CVD) in these population [15]. A chronic inflammation may be the missing link that actually ties protein energy malnutrition to morbiditiy and mortality in these patients. It is interesting that some markers that predict a low risk of CVS events in the general population, such as decerased body mass index (BMI) or lower cholesterol levels, become a strong risk factor for the CVS death in the dialysis patients. The fenomenon of risk factor paradox is caused by the conditions that potentially attenuates the magnitude of protein energy malnutrition, or inflammation [4].

In accordance with this, some studies provided the evidence that the dialysis patients who had gained weight moderately and a larger BMI are more likely to survive [16, 17] Galland et al. [18] showed that more frequent dialysis process increased significantly the body weight and serum albumin of the patients. Rashidi et al. [4] found that increase in dialysis frequency decreased the systemic inflammation and improved the nutritional status if the hemodialysis patients with no influence on the triglyceride, total cholesterol, LDL and HDL cholesterol levels, energy protein and fat intake. In our examination, we observed a significant correlation between CRP values and serum urea levels before and after the hemodialysis procedure. The value of urea after hemodialysis depended on the efficacy of the dialysis procedure itself and the residual renal function. Positive correlation CRP and urea values after dialysis could be explained by the state of the chronic catabolic condition of dialysis patients, or the uremic inflammation [19].

The presence of inflammation seems to be influenced by genetics and/or different cultural habits, since it is a common phenomenon in the European and North American ESRD patients, with lower prevalence in the Asian patients. The same goes to frequency of diabetes mellitus. The frequency od diabetic nephropathy among the ESRD patients population varies from 12% do 50% [4, 11, 20, 21]. The impact of diabetic nephropathy in ESRD in our study was 30.6%. There was no corellation between CRP levels and frequency of diabetes mellitus among our patients. Nevertheless, in the group with the higher CRP values, we observed significantly

more diabetics than in the group with the normal CRP serum levels. In the longitudinal study which included over 2,500 Japanese type 2 diabetic patients, CRP was indipendently associated with future risk for developing nephropathy, but not with the risk of progressing of diabetic nephropathy [22]. In the study of Nath et al. [14] , CRP serum levels were significantly higher and positively correlated with albumin levels in the group od 80 patients with diabetic nephropathy compared to the controls. Diabetes mellitus is a proinflammatory state *per se* that is accompanied with accelerated CVS events and by up to 10-fold elevated CRP levels [8] . Tubular and glomerular CRP staining increases with declining renal function and increasing severity of hystological lesions in the diabetic patients with nephropathy. Still it is independent of proteinuria and it is possibly locally produced [23]. So, an absence of significant corellation between CRP serum levels and diabetic states in our study group as well as with other parameters of metabolic control was somewhat unexpected. The explanation could be found in a small sample size of our examined group.

Conclusion

Our study observed a high rate of inflammation in the dialysis patients presenting as the high frequency ~~of~~ elevated CRP levels in the examined population. Negative correlation between the CRP levels and serum albumin and cholesterole levels, suggests that a chronic inflammation may be the missing link that actually connect protein energy malnutrition with a high morbiditiy and mortality rate in these patients. Interventions that improve nutritional status and reduce a chronic inflammation along with routine measurement of the serum CRP levels may improve the survival rate of dialysis patients.

Acknowledgement

This work was supported by a Project MFVMA/8/15-17.

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Received on September 12, 2016.
Accepted on October 11, 2017.
Online First October, 2017.

ORIGINAL ARTICLE

UDC: 611.13/.14:611.98]::617.58-053.2
https://doi.org/10.2298/VSP170525166S

Anatomic study of septocutaneous system of the human fetuses' lower leg: posterior tibial artery

Anatomska studija septokutanog sistema donjeg ekstremiteta fetusa: *arteria tibialis posterior*

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Abstract

Background/Aim. Lower-leg septocutaneous system of perforating blood vessels represents the vascular basis of fasciocutaneous flaps. Additionally, it is of a particular importance when designing distally based fasciocutaneous flaps which represent the "workhorse" in the reconstruction of the distal third of the lower leg and foot. The aim of this study was to analyse the vascular anatomy of posterior tibial artery and its septocutaneous (fasciocutaneous) perforating arterial vessels. **Methods.** The dissection was conducted on 20 fetuses of both sexes and of gestational age from 20 to 28 weeks. Cluster analysis was applied to the data on vascular anatomy of posterior tibial artery and its septocutaneous performating arterial vessels. **Results.** A total of 212 perforating arterial vessels was identified. The average number of perforating arterial vessels was 5.32 (ranging from 4 to 7). It was identified that septocutaneous perforating blood vessels are more likely to be found at certain levels ("safe levels of finding perforators"). These are: second, third, fifth and sixth tenth (measured as a distance from intermalleolar line to popliteal crease). **Conclusion.** The presence of septocutaneous system of perforating blood vessels and reliability of their localization even in the fetal period allows application of these findings in the lower leg reconstructions in children of early age. It also contributes to the greater level of understanding of anatomy of the lower-leg vascular system. Finally, it provides a basis for understanding the development of this system as it is now possible to compare results obtained on fetuses with those obtained on adults.

Key words:
anatomy; fetus; leg; surgical flaps; tibial arteries.

Apstrakt

Uvod/Cilj. Perforatori septokutanog sistema krvnih sudova potkolenice predstavljaju vaskularnu osnovu fasciokutanih režnjeva. Poseban značaj imaju pri dizajniranju distalno baziranih fasciokutanih režnjeva koji predstavljaju moćnu metodu u rekonstrukciji defekata distalne trećine potkolenice i stopala. Cilj rada bio je detaljno kvantitativno ispitivanje vaskularne anatomije zadnje tibijalne arterije i njenih septokutanih (fasciokutanih) perforatora. **Metod.** Istraživanje je sprovedeno na 20 fetusa oba pola gestacione starosti 20–28 nedelja. Primenom „*Cluster*" analize, obradjeni su podaci dobijeni u ovom istraživanju. **Rezultati.** Ukupan broj septokutanih perforatora iznosio je 212. Prosečan broj perforatnih krvnih sudova (arterijskih) bio je 5,32 (minimum 4, maksimum 7). Istraživanje je pokazalo da je verovatnoća nalaženja septokutanih perforatora bila veća na određenim nivoima (tzv. „sigurni nivoi nalaženja perforatora"). To su: druga, treća, peta i šesta desetina (mereno kao distanca od intermaleolarne linije do zatkolene brazde). **Zaključak.** Prisustvo perforatora septokutanog sistema krvnih sudova i pouzdanost njihove lokalizacije još u fetalnom periodu omogućava primenu ovih nalaza u rekonstrukciji potkolenice i stopala već kod dece. Ovo istraživanje takođe doprinosi dubljem razumevanju anatomije vaskularnog sistema potkolenice. Ono obezbeđuje osnovu razumevanja razvoja ovog

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sistema krvnih sudova i mogućnost poređenja rezultata dobijenih istraživanjima na fetusima sa rezultatima dobijenih kod odraslih.

Ključne reči:
anatomija; fetus; noga; režnjevi, hirurški; aa. tibiales.

Introduction

Discovery of fasciocutaneous blood vessels system of the lower leg by Ponten [1] in the eighties of the last century resulted in more profound understanding of skin vascularization. The main arteries of the body were reexamined with an emphasis on the perforating branches which provide direct skin vascularization. Flaps, that are based on the blood vessels passing through the septum duplication in the lower leg, consist of skin, subcutaneous adipose tissue, and deep fascia and are named fasciocutaneous flaps [2, 3]. They can be either direct or indirect flaps [4]. The initial advancement in this area was followed by a period when numerous studies on this blood vessels system were conducted. This period was marked by two distinct pathways: solving disagreements and confusion in the nomenclature of perforator flaps and further exploration and understanding of new concepts of perforator flaps [5].

From the practitioners' point of view, a discovery of this type of flaps was especially important because of its structural characteristics and lack of reliable flap locations for lower leg area [6]. Patient studies showed that flaps are the best choice when it comes to the lower leg reconstruction. The greatest advantages are the simplicity of the procedure, very high success rate and relatively small number of minor disadvantages. Furthermore, the procedure can be applied to wounds of different origin (e.g., fourth degree burn injuries, blast and high-velocity projectile wounds, distal tibial fracture) and injuries of various size, location and depth [7].

Numerous anatomical studies on cadavers were carried out in order to determine the localization of perforating blood vessels of the main lower-leg arterial trunk. The results, although often significantly different, were of a great help in planning and designing the distally based flaps of the lower leg ("separated asseptocutaneous perforators of the lower leg"). However, studies on fetuses that would shed some light on the vascular anatomy of this system of blood vessels are very rare and often inconclusive, despite the fact that pediatric cases are more complicated [8, 9] and require further theoretical knowledge. The aim of this study was to provide a comprehensive, clear and conclusive overview of the lower-leg septocutaneous system of skin blood supply in fetal age. The results were statistically analyzed in order to enable comparison with other studies of this kind. Implications of this article go beyond contribution to the theoretical knowledge, as the information provided can be applied to cases of lower leg reconstruction in children.

Methods

The study was conducted on 40 lower extremities from 20 human fetuses. The fetuses were prepared by fixation in 10% formalin and blood vessels of 10 fetuses were injected with Micropaque solution (barium sulfate) (Merck, Darmstadt, Germany) for better visualization. Fetuses were collected in the Department of Anatomy between 1962 and 1985. All fetuses were medico-legally obtained from the Clinic of Gynecology and Obstetrics of the Faculty of Medicine in Niš, Serbia. The study was approved by the Ethics Committee of the Faculty of Medicine, University Niš, Serbia (permision from 22.9.2016). No established anatomical deformities or systematic diseases were recorded.

Fetal age ranged from the third to the ninth lunar month and was established by measuring crown-rump length. Microdissection of the fetal lower extremities was performed under 5× magnifying lenses. Two horizontal cuts and one vertical cut of the skin were made (Figure 1). The first horizontal cut was made at the level of the popliteal fold and the second was made at the level of the medial and lateral malleolus (Figure 1). The vertical cut extended from the middle of the upper horizontal cut at the level of the popliteal fold to the middle of the lower horizontal cut. After that, the dissection was going through the skin, subcutaneous tissue, and fascia, and then carefully continued medially and laterally until reaching medial and lateral septum of the lower leg. There, we noted the origin of arterial septocutaneous vessels from the posterior tibial artery. Also, we could clearly measure the distance from the lower horizontal cut to the origin of perforating vessels. Characteristic cases were photographed (Figure 1).

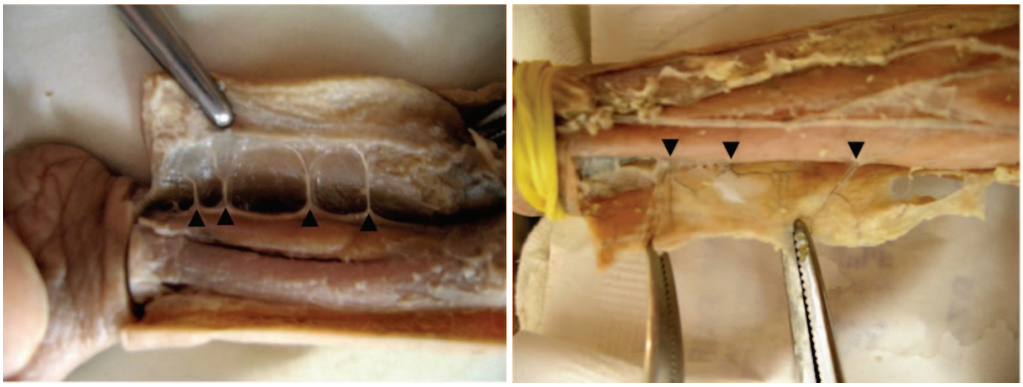

Fig. 1 – Posterior tibial artery perforators (fetal dissections with injected contrast).

Data analysis

Cluster analysis was conducted on the raw data obtained from the primary research. The portable IBM SPSS Statistics v19 was used. K-means cluster analysis was conducted. Due to the small data set, the number of iterations was set at 10 in order to determine whether it is possible to run this type of analysis. The SPSS conducted the command without any further notifications, meaning that the data set is large enough. The number of clusters was set at three and clusters were divided on the basis of the number of perforators found in each area of fetuses' lower leg. Clusters were divided into the low, medium and high-density area. Additionally, the cluster membership information was saved.

Results

The anatomical microdissection of fetal lower legs with (Figure 1) or without contrast injected (Figure 2) was used in order to define the number of septocutaneous perforator vessels and their localization.

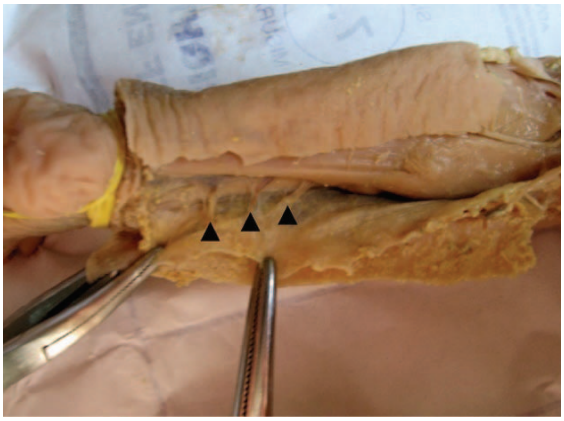

Fig. 2 – Posterior tibial artery perforators (without contrast) from their origin to the deep fascia.

The examination of septocutaneous perforating vessels of posterior tibial artery confirmed the existence of 4 such vessels in 6 dissections and 5 in 20 dissections. Furthermore, there were 6 perforating vessels in 9 dissections and in 5 dissections their number was 7 with an average value of 5.32 and with the standard deviation of 0.88 (total of 212 perforating vessels). The septocutaneous perforating vessels of posterior tibial artery originated between the flexor digitorum longus muscle and soleus muscle.

In order to statistically process the data, conduct the subsequent analysis and enable comparability of the results obtained by our research and the results of other similar studies, the fetal lower legs were "divided" into 10 equal parts.

The levels at which perforating blood vessels were encountered as well as the outbreak schedule of perforating vessels of posterior tibial artery and peroneal artery, obtained by dissection of the fetuses, were counted and shown in Table 1.

Finally, the K-means cluster analysis was performed on the data set and the variable of interest was the number of dissections where the perforating vessels of septocutaneous perforating vessels of posterior tibial artery were found (labeled Variable 1). The analysis conducted on Variable 1 showed that, on average, 4 septocutaneous perforating vessels were found in the cluster 1 (M = 4), 33 were found in the cluster 2 (M = 33) and 22 were found in the cluster 3 (M = 22). The analysis also showed that the cluster 2 was represented with 4 cases while the cluster 1 and 3 consisted of 3 cases each. The cluster membership showed that the high density cluster was the cluster 2. The low-density areas (cluster 1) were the first, ninth and tenth 1/10 of the lower leg. The medium-density areas (cluster 3) were fourth, seventh and eighth 1/10 of the lower leg, while the high-density areas were the second, third, fifth and sixth 1/10.

Additionaly, the independent samples *t*-test was used in order to determine whether there was a statistically significant difference between distributions of dissections in the areas where perforators were found as observed in adults (in our previous study [10]) and fetuses. Age was used as a grouping variable with the value 1 assigned to adults and value 2 assigned to fetuses. As the number of adult cadavers was higher than the number of fetal cadavers, instead of using absolute values, the test was run on the percentages of the total number of dissections for each of the ten areas.

Table 1

Number (and percentage) of fetuses with the septocutaneous perforating vessels of posterior tibial artery and their percentage per each group

Septocutaneous perforating vessels*	Dissections where perforators were found (n)	Total number of dissections (%)	Total number of perforators (%)
First	5	12.25	2.36
Second	36	90	16.98
Third	32	80	15.09
Fourth	19	47.5	8.96
Fifth	35	87.25	16.51
Sixth	30	75	14.15
Seventh	19	47.5	8.96
Eighth	27	67.5	12.73
Ninth	6	15	2.83
Tenth	2	5	0.9

***1/10 length of the lower leg (from intermalleolar line to popliteal crease).**

The results of the *t*-test showed that there was no statistically significant difference between adults and fetuses (t = 0.146, p = 0.866), which means that the distribution of dissections in ten areas where perforators were found was highly similar for the fetal and adult cadavers.

Discussion

The lower leg area has been a focus of interest of many different research papers and extensive studies. However, knowledge about this body area is still far from complete. While the importance of the topic was recognized, it is still fairly insufficiently researched (for example, the posterior lower leg skin and its vascular anatomy [11–13] illustrate this statement). Implications of such theoretical gaps extend into the practice. Consequentially, numerous challenges arise, such as often quoted poor success rates of lower leg soft tissue reconstruction [14]. A step forward in this area was made with the recognition of importance of faciocutaneous perforators and flaps. Technology development was one of the biggest obstacles and once when color duplex imaging was introduced, it would become feasible to access deep fascia [7]. Ponten [1] was the first to recognize potential of fasciocutaneous flap when it comes to surgical solutions for soft tissue defects. After that, a long stream of researches followed. Hupkens et al. [13] conducted an anatomical study to localize and classify the lateral lower leg perforators. The same author gave his contribution by researching the medial lower leg perforators, their distribution and characteristics. Apart from anatomical studies focused on the general characteristics, a number of published papers revolved around soft tissue reconstruction and the role of fasciocutaneous flaps in the reconstruction process [7, 14–16]. While it is evident that this area of research is becoming more complex and compatible with practical needs, a vast majority of studies was conducted on adults. The studies conducted on children are quite rare (the study by Whaib [9] is one of the very few ones exploring reconstruction of full thickness of soft tissue defect of lower extremities in children [9]) but still more present than the studies on fetuses. We identified a lack of studies conducted on fetuses as a serious research gap. A study undertaken by Ugrenović et al. [17] on neurovascular stalk of the superficial sural flap is one of the few ones conducted on fetuses.

We identified several reasons why this research gap should be bridged. The septocutaneous system of lower leg perforators is already very well developed in the fetal period. There is a rather clear clustering pattern of perforators at different levels which reflects the pattern we find in adults. Having this in mind, as well as the fact that this system represents the vascular basis of fasciocutenous flaps, we can conclude that the skin and soft tissue defects of distal lower leg area can be taken care of during early childhood by implementing this reconstructive method [18–20]. The significance of this research lies in the fact that now pre-operative mapping and finding perforators using ultrasound is significantly easier (and it is almost not necessary at all), because the operative method requires presence of at least one reliable perforator and in this study we showed the exact levels of emergence of the most distal perforators. Furthermore, this paper also contributes to increasing the level of understanding of anatomy of the lower-leg vascular system.

The independent samples *t*-test, applied to results of our research obtained in fetuses and adults, confirmed that there is no statistically significant difference between adults and fetuses, which means that the distribution of dissections in ten areas where perforators were found is highly similar for fetal and adult cadavers.

Finally, our research provides a basis for understanding the development of this system as it is now possible to compare results obtained on fetuses with those obtained on adults. Despite the well-known fact that during embryogenesis and fetal development significant changes occur in the number and caliber of the lower leg vascular vessels as well as obliteration of some blood vessels and creation of new ones, the basic model of blood vessels pattern of this system stays rather stable until its final stage of development.

Results of this study is not only of academic importance but also of practical one. Lower leg defects in children can be posttraumatic (motor vehicle accidents, burns, war injuries) or postoperative (after tumor removal). Many factors should be considered in reconstruction of this kind of defects in children. In addition to the lack of children cooperation postoperatively and functional requirements, a surgeon must be aware of anatomical limitations, such as small structures and state of growth and developments of vascular system of the lower leg.

Results obtained in this study clearly show that there is exactly defined the schedule outbreak of septocutaneous perforators of posterior tibial artery.

Conclusion

Despite the fact that sample size for this kind of analysis is not as huge as it could be, it is sufficient in a way that we can draw some important conclusions: the septocutaneous system of posterior tibial artery is well- established in late fetal age already; there is the precisely defined schedule of septocutaneous perforator's outbreak which is very similar to the pattern we found in adults (there is no statistical difference between these two study groups).

All this means that a reconstructive surgeon can safely use this data to plan reconstruction of almost any kind of the lower leg and foot defects in children, using the fasciocutaneous flaps (perforator flaps, flaps with reverse flow etc.) whose vascularisation relies on the septocutaneous system of vessels.

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Received on December 15, 2016.
Revised on March 02, 2017.
Accepted on March 03, 2017.
Online First March, 2017.

GENERAL REVIEW

UDC: 616.9:616.36-002
https://doi.org/10.2298/VSP170815159P

Current knowledge on Hepatitis E virus infection

Aktuelno znanje o hepatitis E virusnoj infekciji

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Key words:
diagnosis; disease outbreaks; epidemiology; hepatitis e; hepatitis e virus; prevalence; therapeutics.

Ključne reči:
dijagnoza; infekcija, putevi širenja; epidemiologija; hepatitis e; hepatitis e, virus; prevalenca; lečenje.

Introduction

Hepatitis E is an emerging viral disease affecting both humans and different kinds of domestic and wild animals. In developing countries, human hepatitis E virus (HEV) has a trend for epidemic spread with benign outcome, except pregnant women [1]. The death rate due to HEV exceeds 25% in the third trimester [1]. In developed countries, the autochthonous cases of human HEV infection are associated with a consummation of poorly heat-treated meat and meat products (mostly domestic and wild swine) [1].

The current article presents systematic analysis of the epidemiology, etiology, clinical signs, diagnosis, therapy and prevention of HEV infection.

History

Hepatitis E is "recognized" in 1980 during the epidemic in the valley of Kashmir (India) [2]. The affected people were between 11–40 years old, native citizen of the valley with common source of water [2]. The infected area was characterized by a high level of viral distribution and mortality among pregnant women [2]. The epidemic spread, the incubation period, clinical signs and biochemical results of the examined patients were similar to manifestation of Hepatitis A virus infection [2]. A few months later Wong et al. [3], published results from retrospective serological study of stored samples from a large hepatitis epidemic in Delhi, India (1955–1956) and two smaller infected areas in Ahmedabad, India (1975–1976) and Pune, India (1978–1979). The results from that study established a few cases of acute hepatitis B and none acute hepatitis A [3]. Owing that fact was given the idea for existence of „non-A, non-B hepatitis agent" [3]. The next serious breakthrough was in USA (1997), when was found a swine virus, named „swine hepatitis virus" [4]. At the same time, the first case of human HEV was described. The isolated virus had similar genomic characteristics to the swine HEV [5, 6]. That disclosure determines the zoonotic character of the virus [1].

Etiology

HEV belongs to the family of *Hepeviridae,* genus *Hepevirus* [7]. According to the current classification, the family *Hepeviridae* is divided into two genera: *Orthohepevirus* and *Piscihepevirus*. *Orthohepevirus* includes four species [8]: *Orthohepevirus A*: isolated from human, swine, deer, mongoose, rabbit, camels; *Orthohepevirus B*: isolated from birds; *Orthohepevirus C*: isolated from rats, a big Indian rat, Asian kind of mole, ferret and mink; *Orthohepevirus D*: isolated from bats.

Until now, there are four main genotypes, with more than 24 subtypes and only one serotype [9–11]. Genotypes 1 and 2 (HEVgt1 and HEVgt2) are linked with large human epidemics in countries with poor hygiene [12]. Genotypes 3 and 4 (HEVgt3 and HEVgt4) infect humans and other mammals, which cause sporadic cases of Hepatitis E in industrialized countries [12].

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HEV is a small virus with a diameter approximately 27 to 32 nm, icosahedral symmetry, spherical shape and simple structure [12]. The virion contains single positive-stranded RNA with size 7.2–7.5 kb [13]. HEV genome includes 5′ untranslated region (UTR), three opened-reading frames (ORF1, ORF2 and ORF3) and 3' UTR, followed by poly-A tail [13]. Each reading frame has different functions [12, 13]: ORF1: is situated next to 5′ and encodes unstructured proteins with enzyme function (methyltransferase, papain-like cysteine protease, macrodomain, helicase and RNA-dependent RNA polymerase); ORF2: is situated next to 3′ and encodes viral capsid protein, build from 660 amino acids, which is responsible for the viral cutting, interaction with target cells and immunogenicity properties; ORF3: encodes small protein, build from 113–114 amino acids, which is responsible for replication and building cytoskeleton and it also decreases inflammatory response and protects viral-infected cells.

Epidemiology and prevalence

Hepatitis E is an endemic disease in Central and Southeast Asia, in tropic and subtropical countries in Africa and Central America [1]. In the endemic area, large waterborne epidemics were described. Hepatitis E is sporadically reported in the USA and Europe [1]. In the developed world, it was thought that the infection was associated with traveling to the endemic regions [14]. But nowadays, it is known that it is a local, autochthonous transmission.

HEVgt1 and HEVgt2 are responsible for enterically-transmitted epidemics in tropical and some subtropical areas [15]. They are associated with contamination of water (water supplies) and poor sanitation conditions [15]. Both genotypes cause acute hepatitis in humans.The virus is found in feces, an environment contaminated with human's feces [15]. A study in Uganda showed that environmental factors could be much more important for transmission, than it was thought [16]. In nonendemic regions, the mechanisms of transmission are much less known, in contrast to endemic areas the contaminated water is a documented source of infection [17]. HEV is the only one among other hepatotropic viruses with zoonotic character and animal reservoir [17]. The literature search presents that most of the autochthonous human cases are associated with the consumption of raw and undercooked meat infected with HEV [18–20]. Pigs are considered to be the main reservoirs for HEVgt3 and HEVgt4, and the two genotypes are found in pigs all over the world [21]. Antibodies against HEV are found in chickens, dogs, rodents, cows, sheep, goats, monkeys and other animals [22]. HEVgt3 is responsible for most of human HEV infections in Europe, North America and East Asia [23]. HEVgt3 dominated in swine samples in Europe and America. The virus was detected in pork products [19, 24]. Strains of HEVgt3 were recently found in pigs in Africa [25]. In 1998 HEVgt4 was responsible for sporadic human HEV cases in Taiwan and after that the virus was found in pigs at the same geographical area [26, 27]. In China, HEVgt4 is the most common virus in humans and swine [28, 29]. Also, it is endemic in Japan [30]. In Europe, Japan and USA, specific antibodies against HEV are often detected in domestic pigs, which prove their role as a source of HEV infection [31, 32]. Studies done in Japan and France presented the transmission of the virus through a consumption of meat and sausages, made of domestic pigs, wild boars and deer [19,33]. Acute hepatitis E was described after eating pork meat infected with HEVgt4 in Japan [34]. In Japan, it was reported a severe human case after eating raw liver from a wild boar, whereas in Europe, the severe human infections were related to consumption of pork meat [19, 35]. The phylogenetical analysis of HEV samples from Japan indicated a previous transmission of the virus from domestic pigs to wild boars [36]. Urine was identified as a possible source for swine HEV infection [1]. It was established that swine HEV could pass colostrum, while transplacental transmission is arguable [1]. Another possible way of HEV transmission is the direct contact of people and swine [1]. The serological studies in the USA reported that veterinaries and people, who are working in slaughterhouses had high positive results for anti-HEV IgG compared to population with lower risk for direct contact with pigs and pork products [37]. A higher rate of seroprevalence was found among foresters in comparison with the seroprevalence among blood donors [1, 21]. HEV infection could be transmitted by transfusing blood and blood products [38]. Swine products, such as swine heparin and others, used in human medicine, could be a risk factor for HEV spread [39]. Other possible risk for HEV source could be feces or manure [40]. A study reported the presence of HEV in the manure storage facilities [40].

Nowadays, wild boars are thought to be an important natural reservoir for HEVgt3 and HEVgt4 [1]. The recent study done across Asia and Europe showed a high rate of HEV seroprevalence likewise a molecular evidence of HEV infection in wild boars [1, 12, 21]. Takahashi and Okamoto [41] found HEV RNA in 1.1%–13.3% of the examined wild boars and seropositivity varied between 4.5% to 34.4%. In Germany, wild boars are considered to be one of the main sources for HEV transmission [42]. HEV RNA could be found in the serum, gall and liver from wild boars [43]. HEV samples collected from the wild boars showed great genetic variability [9, 11].

In many European countries, different serological studies for human HEV seroprevalence were conducted over the past years. We present the results of HEV seropositivity in blood donors from 24 studies (Table 1) [44–67]. The calculated mean ± standard deviaton (SD) human HEV seroprevalence is 15.21 ± 14.20 (95% confidence interval (CI) = 12.61–43.04). The great variety of positive results are affected by geographic location, national traditions and customs, design of the study, year of projects conducted and type of diagnostic tests. Regardless of the published diversity, these data confirmed the seroprevalence of HEV among blood donors in different European countries.

Worldwide, the main animal reservoir for HEVgt3 and HEVgt4 are domestic pigs and wild boars [1, 68]. Data for swine HEV seroprevalence in European countries are summarized in Table 2 [32, 67, 69–78]. There is a broad spectrum of variety in seropositivity among different countries. The evaluated mean ± SD swine HEV seroprevalence is 47.93 ± 19.75 (95% CI = 9.23–86.64). The presented average percentage for seropositivity illustrates the existence and persistence of the virus among pigs and their potential animal reservoir.

Table 1

Seroprevalence of hepatitis E virus (HEV) in blood donors (BD) in European countries

References study	Country	Year of publication	Investigated BD (n)	HEV positive BD (%)
Macedo et al. [44]	Portugal	1998	50	4.0
Tarrago et al. [45]	Spain	2000	863	2.9
Olsen et al. [46]	Sweden	2006	108	9.3
Boutrouille et al. [47]	France	2007	1998	3.2
Dalton et al. [48]	England	2008	500	16-25
Mansuy et al. [49]	France	2008	529	16.6
Christensen et al. [50]	Denmark	2008	169	20.6
Mansuy et al. [51]	France	2011	512	52.5
Kaufmann et al. [52]	Switzerland	2011	550	4.9
Dremsek et al. [53]	Germany	2012	301	11.0
Fogeda et al. [54]	Spain	2012	2305	1.08
Cleland et al. [55]	Scotland	2013	1559	4.7
Slot et al. [56]	Netherlands	2013	5239	26.7
Juhl et al. [57]	Germany	2014	1019	6.8
Petrovic et al. [58]	Serbia	2014	200	15.0
Fischer et al. [59]	Austria	2015	1203	13.55
Holm et al. [60]	Denmark	2015	504	10.7
Mansuy et al. [61]	France	2015	3353	39.1
Puttini et al. [62]	Italy	2015	132	9.1
Aydin et al. [63]	Turkey	2015	327	0.92
Ricco et al. [64]	Italy	2016	199	7.0
Mansuy et al. [65]	France	2016	10569	22.4
Lucarelli et al. [66]	Italy	2016	313	49.0
Lange et al. [67]	Norway	2017	1200	14.0

Table 2

Seroprevalence of swine hepatitis E virus (HEV) antibodies in European countries

References study	Country	Year of publication	Investigated pigs (n)	HEV positive pigs (%)
Savuta et al. [69]	Romania	2007	145	42.7
Savuta et al. [70]	Romania	2008	69	49.27
Asimoula et al. [71]	Greece	2009	96	80.0
Lupulovic et al. [72]	Serbia	2010	315	34.6
Martinelli et al. [73]	Italy	2011	1422	50.21
de Oya et al [74]	Spain	2011	1141	20.4
Krumbholz et al. [32]	Germany	2013	2273	46.9
Connor et al. [75]	Ireland	2015	330	27.0
Weiner et al. [76]	Poland	2016	143	44.1
Lange et al. [67]	Norway	2017	153	90.0
Caruso et al. [77]	Italy	2017	879	50.0
Pishmisheva et al. [78]	Bulgaria	2017	85	40.0

Clinical manifestation

The most common clinical manifestation of HEV among people in the endemic areas is acute icteric hepatitis with typical clinical and laboratory signs [14]. Sometime prolonged cholestasis could be developed or asymptomatic infection may occurred [14]. A high rate of fulminant hepatic failure and death were mentioned among pregnant women in the hyperendemic areas [79, 80]. In nonendemic areas, the virus affected mainly elderly men, people with accompanying liver diseases and alcohol abuse [81–83]. The autochthonous cases could be manifested as an acute hepatitis, asymptomatic infection, and nonspecific symptoms with anicteric diseases [48]. In contrast, the severe illness does not manifest during pregnancy in the nonendemic regions [81, 82]. Chronic HEV infection was described in solid-organ transplant recipients, patients with hematological diseases, HIV patients, people under immunosuppressive conditions and anticancer chemo-

therapy [81, 82, 84–86]. In such case, the liver biopsy illustrated liver fibrosis, which predicts the progress to cirrhosis [87].

Swine HEV infection does not present typical clinical symptoms and signs. Usually animal diseases are characterized with fluctuations in body temperature or body weight [1]. After a subclinical HEV infection, mild microscopic lesions in the liver could be developed [11]. The pathological findings include viral antigen in the hepatocytes, positive immunohistochemical changes in the small and large intestines, lymph nodes, tonsils, spleen and kidneys [12]. A Spanish study reported no correlation between HEV RNA and the histological changes in the liver [88]. So it is arguable whether or not a natural HEV infection causes any histological changes in the liver.

Laboratory diagnostics

The most common method for routine diagnosis of HEV infection is the serological examination. The laboratory diagnostics use serum samples for detection of HEV antibodies by enzyme-linked immunosorbent assays (ELISA) and western blot assays [1]. The tests estimate the presence of antibodies of class IgM and IgG (rarely IgA) against HEV. In the first stage of the infection, antibodies of class IgM appear and mark acute present infection [1]. After that antibodies of class IgG follow up and show a recent or past infection. The serum samples are collected for the serological tests in humans, for the pig examination it could be collected sera or meat juice [89]. In humans, anti-HEV IgM levels peak around the time of the alannine aminotransferase (ALT) peak and may persist up to 5 months after the onset of the illness (Figure 1) [14]. A little later anti-HEV IgG begin to produce, they remain during the acute phase, the recovalescent period and also maintain high levels at least one year after the recovering (Figure 1). Commercially available immunoassays differ substantially in their sensitivity and specificity and the false-positive results varying from 0.3% to 2.5% [90, 91].

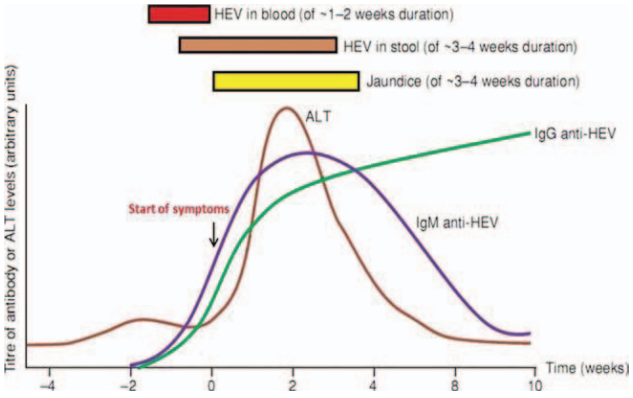

Fig. 1 – The development of hepatitis E virus (HEV) infection in humans.

The majority of pigs are naturally infected with HEV at the age of 2 to 4 months [4]. Eighty six percentage of pigs are naturally infected with the virus until their eighteenth week [92]. The maternal antibodies decline at the age of 8 to 10 weeks [17]. After the reduction of them, the piglets could be attacked by the virus around the second week after birth [11]. The swine HEV infection is accompanied with a transient viremia lasting 1 to 2 weeks and a fecal-oral emission of the pathogen continuing three to 7 weeks [9]. The number of viremic pigs increase from 9 weeks with peaking around 15 weeks, following decline to slaughter age [93]. Seroconversion of anti-HEV IgM, which is related to the peak of the virus excretion through feces, is followed by seroconversion of anti-HEV IgG with the highest concentrations at the age of 4 months (Figure 2) [17]. Interestingly enough, the presence of antibodies does not always assure the absence of the virus because HEV RNA and the anti-HEV antibodies are found in pigs together. This leads to the conclusion that these animals are HEV-reservoirs [21].

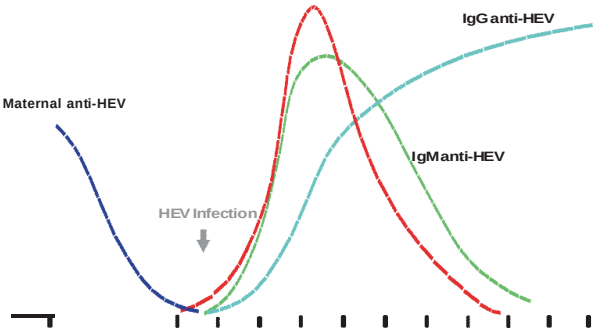

Fig. 2 – The course of a typical swine hepatitis E virus (HEV) infection.

Nowadays, the detection of HEV RNA using molecular-genetic methods is considered as the "golden standard" in the laboratory diagnosis [1]. The detection of RNA is performed by different RT-PCR methods, amplifying genomic fragments in one of the three ORFs [11, 21]. HEV RNA could be found in the patients' blood and/or feces in the prodromal period, and after that, the virus could be detected in feces for another 2 weeks [12]. The viremic period is very short, therefore HEV RNA could not be always found in sera. The presence of HEV RNA is a definitive marker for a current infection.

Recently, the establishment of HEV antigen is introduced as an early diagnostic method [94, 95]. However, the test has a low sensibility compared to the methods that use the amplification of nucleic acid [96]. The presence of HEV antigen in different swine tissues using immunohistochemistry was recently demonstrated [97]. The detection of HEV antigen in the liver tissue representing a valuable tool for the viral establishment in biopsy, autopsy and explant liver tissues [98].

Therapy and prophylaxis

There is no specific therapy for the acute HEV infection in humans, because in most cases the illness is self-limiting. The management of acute illness in the immunocompetent patients include a strict diet, administration of fluids and hepatoprotective medications. In case of acute liver failure intensive care treatment is required and sometime liver transplantation is needed [99]. In chronic HEV infection, the

administration of pegylated interferon alpha-2a/alpha-2b, or ribavirin for 3–12 months were applied as a specific antiviral therapy [100, 101]. A recombinant vaccine showed 94%–100% efficacy in a phase III study of > 100,000 Chinese adults [102]. The vaccine protected from HEVgt1 and HEVgt2 [102].

There are no specific therapeutic medications for animals. The swine HEV vaccine has not been developed yet.

The prevention measures are guided to improving sanitation and hygiene in developing countries [17]. In developed countries, the population with a high risk could be informed about the virus and his zoonotic characteristic, and, consequently, should be asked to reduce and/or avoid consumption of raw, or undercooked meat and meat products from pigs, wild boars, deer and direct contact with infected animals.

Conclusion

Swine are defined as the main reservoirs for the zoonotic HEVgt3 and HEVgt4. The infection is widely spread in pigs all around the world. Just like in humans, the fecal-oral mechanism of the transmission is thought to be the main one in animals as well. The nature course of swine HEV is subclinical manifestation, therefore the sick animals are hard to be focused and isolated. The animal reservoir and the lack of specific prophylaxis transform HEV as a potential threat to public health.

Conflict of interest

None.

R E F E R E N C E S

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Received on August 15, 2017.
Accepted on October 10, 2017.
Online First November, 2017.

CASE REPORTS

UDC: 616.411-056.7:616.155.2
https://doi.org/10.2298/VSP170110168M

Ultrastructural and morphometric analysis of enlarged platelets in congenital isolated asplenia

Ultrastrukturna i morfometrijska analiza uvećanih trombocita kod bolesnika sa izolovanom kongenitalnom asplenijom

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Abstract

Introduction. Congenital asplenia is an extremely rare condition that can be separate entity due to a specific defect of spleen development or may occur in the context of a malformation syndrome. The patients with asplenia have thrombocytosis and susceptibility to life-threatening infections. **Case report.** We report a 52-years-old female patient with isolated congenital asplenia with pseudothrombocytopenia and giant platelets. Estimation of platelets life with radioactive indium showed normal lenght of platelets life (9 days). Flow cytometric analysis of platelets showed normal expression of CD41 and CD42b antigens. The mean platelet diameter of asplenic patient measured on the ultrathin sections by the transmission electron microscope was significantly higher than in the healthy individuals (3.81 ± 1.16 μm vs. 2.37 ± 0.61 μm, $p < 0.05$). There were very few platelets of diameter more than 4 μm found in healthy individuals (around 1%) in comparison to > 40% of the patient's platelets. The ultrastructural studies revealed normal morphology of megakaryocytes. The platelets were uniformly spheroid in shape with conspicuous pseudopodia and the centralization of granules. There were no marginal bands of microtubules inside the platelets. **Conclusion.** The first case of congenital asplenia with the pseudothrombocytopenia and giant platelets is presented. We discussed the pathogenesis of giant platelets and possible relation of observed ultrastructural changes of platelets with the severe three-vessel coronary artery disease in our patient.

Key words:
blood platelet; congenital abnormalities; microscopy, electron; myh9-related disorders; spleen.

Apstrakt

Uvod. Kongenitalna asplenija je izuzetno retka. Može se javiti izolovano (specifični defekt u razvoju slezine) ili u sklopu malformacionog sindroma. Bolest se najčešće dijagnostikuje u dečjem dobu, a odlikuje je nalaz trombocitoze sa malim tromboctima i infekcije inkapsuliranim mikroorganizmima koje mogu ugroziti život bolesnika. **Prikaz bolesnika.** Prikazali smo 52-godišnju bolesnicu sa izolovanom kongenitalnom asplenijom i pseudotrombocitopenijom sa gigantskim trombocitima. Ispitivanje radioaktivnim indijumom pokazalo je normalnu dužinu života trombocita (9 dana), a protočna citometrija normalnu ekspresiju CD41 i CD42b antigena na trombocitima bolesnice. Srednji dijametar trombocita meren transmisionom elektronskom mikroskopijom (TEM) bio je značajno veći nego kod zdravih osoba (3,81 ± 1,16 μm vs. 2,37 ± 0,61 μm, $p < 0.05$). Kod zdravih osoba bilo je prisutno samo nekoliko trombocita dijametra većeg od 4 μm, (oko 1%), a kod bolesnice je takvih trombocita bilo > 40%. Ultrastrukturna analiza (TEM) pokazala je normalnu morfologiju megakariocita. Trombociti u perifernoj krvi i kostnoj srži bili su uniformno sferoidnog oblika sa vidljivim pseudopodijama, centralizacijom granula i bez vidljive ivične spirale mikrotubula. **Zaključak.** U dostupnoj literaturi nema objavljenih slučajeva kongenitalne asplenije sa pseudotrombocitopenijom i gigantskim trombocitima. Diskutovana je patogeneza gigantskih trombocita i moguća povezanost uočenih ultrastrukturnih promena trombocita sa teškim oblikom trosudovne koronarne bolesti kod prikazane bolesnice.

Ključne reči:
trombociti; anomalije; mikroskopija, elektronska; myh9-povezani poremećaji; slezina.

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Introduction

Congenital asplenia is very rare condition (1 case in 20,000 live births) which occurs sporadically, but also may have family association [1]. Congenital asplenia can be a separate entity due to a specific defect of spleen development, or may occur in the context of a malformation syndrome [2]. Since the spleen is a major producer of antibodies and splenic macrophages have a major role in bacterial phagocytosis, the patients with asplenia or hyposplenia are susceptible to life-threatening or fatal septicemia caused by encapsulated pathogens [2–4]. Life-threatening infections usually occur in childhood, but they were also described in the adult patients [2–4]. All cases with isolated congenital asplenia published so far had thrombocytosis [5–7] together with a common finding of small platelets [2].

The platelet production represents the final stage of megakaryocyte development [2]. It is commonly accepted that during the final stages of differentiation, megakaryocytes extend cytoplasmic protrusions referred to as proplatelets [8]. Proplatelets branch long processes that extend from mature megakaryocytes into the sinusoidal blood vessels of the bone marrow. The proplatelet formation is dependent on the function of microtubules [9]. Microtubular coils similar to those observed in the blood platelets can be detected only at the ends of proplatelets and not within the platelet-size beads found along the length of proplatelets [10]. Thon and Italiano [11] recently identified a previously unrecognized intermediate cell, which they termed a preplatelet. Preplatelets are defined as discoid cells, anucleate platelet progenitors 3–10 μm across that retain the capacity to convert into barbell-shaped proplatelets [11, 12]. These preplatelets may be related both to the young (reticulated) platelets associated with the increased RNA content, and the large platelet commonly seen in inherited/acquired macrothrombocytopenias [11, 12]. Preplatelets reversibly convert into barbell-shaped proplatelets in the blood that divide to form two platelets [11, 12]. When the number of peripheral microtubules is increased, the spectrin-based membrane skeleton becomes disassembled and preplatelets turn out to be incapable of undergoing further barbell proplatelet conversion, resulting in the terminal platelets of a larger size [11, 12]. Macrothrombocytopenia may therefore represent a failure to convert the preplatelets to barbell-proplatelets [11, 12].

We reported a unique case of isolated congenital asplenia presented with pseudothrombocytopenia and enlarged platelets. The patient also had severe coronary heart disease, with no history of life-threatening infections, or bleeding. The particular aim of this case report was ultrastructural and morphometric analysis of enlarged platelets obtained from the patient's peripheral blood and the bone marrow.

Case report

A 52-year-old woman referred to the Hematology Department in 2003 because of pseudothrombocytopenia. She had a 5-year history of arterial hypertension and 2-year history of depressive neurosis. There was no history of abdominal surgery, infections, thrombosis or bleeding. She had no history of prolonged menstrual bleeding. Her two sons had normal number of platelets, normal spleen on abdominal ultrasonography and absence of giant platelets in peripheral blood smear. Physical finding was normal. Full blood counts and white cell differential were in a normal range, except for the platelet number which appeared to be low (54×10^9/L) and the mean platelet volume which was high (14.1 fL). However, when the platelets were analyzed using the CD61 (GPIIIa) MoAbs (ImmunoPLT method), or when they were counted in chamber, the platelet number was found to be around 100×10^9/L. On blood smear, the platelets were unusually large and the Howell-Jolly bodies were present in the red blood cells. The platelet aggregation in response to ristocetin, adenosine diphosphate (ADP), thrombin receptor-activating peptide (TRAP) and adrenalin as well as prothrombin time, activated partial thromboplastin time (aPTT) and bleeding time were normal. The platelet survival, analyzed by radioactive indium, showed normal life span (9 days) with the decreased index of production of platelets in the bone marrow (0.24, normal 1.0). The flow cytometry analysis of platelets showed the normal expression of the CD42b and CD41 antigens. The bone marrow aspirate and trephine biopsy showed mild hypercellularity, normal number of megakaryocytes, mild reactive changes without signs of malignant infiltration. The routine biochemistry and thyroid hormones were in the reference range. The immunological tests [antinuclear antibody (ANA), rheumatoid factor (RF), antibodies against cardiolipin and β_2-glycoprotein] and lupus anticoagulant were negative. The cytogenetic analysis showed normal 46, XX karyotype. Radiography of the chest showed a normal finding. The spleen was not visible by abdominal ultrasonography and computer tomography of abdomen was unremarkable. Scintigraphy using the 99mTc-labeled red cells showed the absence of splenic tissue in abdominopelvic cavity.

The ultrastructural analysis of platelets was done by the transmission electron microscope (TEM, FEI Morgagni 268D) and showed abnormal morphology of platelets, both in peripheral blood and in the bone marrow (Figure 1A-D). The platelets were uniformly spheroid in shape with conspicuous pseudopodia and the centralization of granules (Figure 1D). There were no marginal bands of microtubules inside the platelets (Figure 1D). Megakaryocytes of normal morphology were found on the ultrathin sections of bone marrow aspirates (Figure 1E). The platelets in the bone marrow were also large, spheroid with numerous pseudopodia and the centralization of granules with no signs of dilatation of open canalicular system in the majority of them (Figure 1F-H).

Very few platelets of diameter more than 4 μm were found in healthy individuals (around 1%) in comparison to > 40% in the patient's platelets. The mean platelet diameter (MPD) of asplenic patient from the peripheral blood sample was 3.81 ± 1.16 μm (range: 1.53–6.69 μm) and was significantly higher than MPD in healthy individuals (2.37 ± 0.61 μm, range: 1.01–3.68 μm) ($p < 0.05$, Student *t*-test). The MPD of platelets from the bone marrow aspirate (3.76 ± 0.81 μm, range: 2.09–5.84 μm) was quite similar to MPD from peripheral blood.

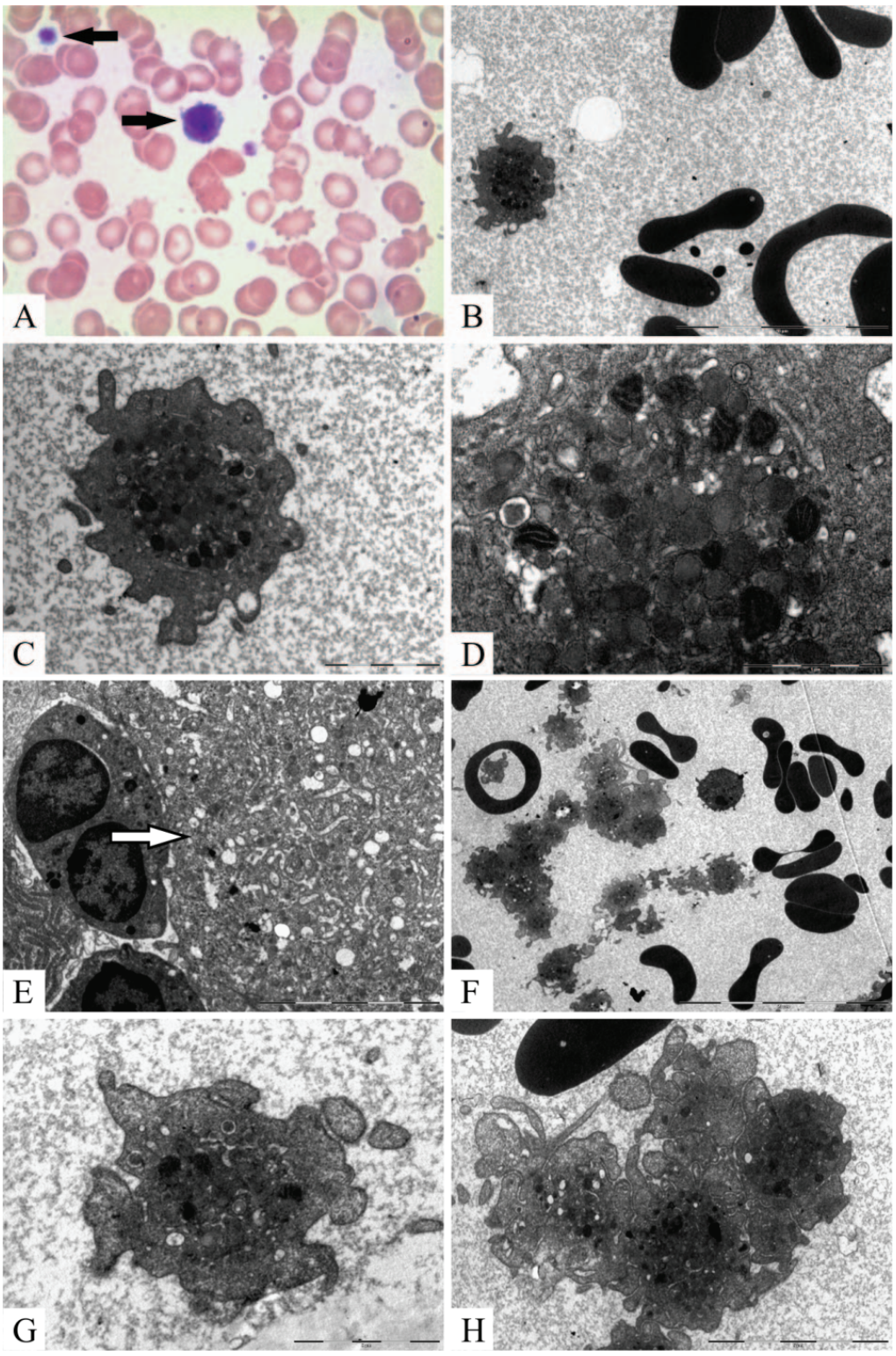

Fig. 1 – Morphology of platelets seen on light microscope (A), and the transmission electron microscope (B-H) in peripheral blood (A-D) and the bone marrow (E-H). (A) Blood smear of the patient studied with the unusually large platelets (arrows) and Howell-Jolly bodies in erythrocytes (May Grünwald Giemsa, x100) (B, C). The spheroid forms of large platelets with pseudopodia without obvious dilatation of open canalicular system. The vacuoles and darker inclusions could be seen in erythrocytes B) x4,400; C) x11,000). (D) A large magnification of platelet shows centralization of granules in peripheral blood and the lack of the visible marginal band of microtubules (x28,000). (E) The ultrastructural appearance of the cytoplasm of megakaryocyte found on ultrathin sections of bone marrow aspirates (x7,100). (F-H) The large, spheroid platelets in the bone marrow with numerous pseudopodia and the centralization of granules although no sign of dilatation of open canalicular system could be spotted [F) x2200; G x14,000; H) x7,100].

In 2009, a pacemaker was implanted in the patient because of tachybrady form of atrial fibrillation. In July 2012, she complained on chest pain and fatigue. Coronarography revealed the three-vessel coronary artery disease. The urgent surgical revascularization of the heart was successfully done, followed by the anticoagulant therapy. On her last follow-up (April 2016) she was in a good clinical condition and without complains.

Discussion

Isolated congenital asplenia is a rare condition. Literature review [2] only yielded about 50 cases since the first report of Myerson and Koelle [13] in 1956. This report described 24 sporadic and 26 family cases of isolated congenital asplenia, a majority in children. Our patient did not have other somatic anomalies beside asplenia and this condition was not found in any family member. Therefore, we concluded that our patient had sporadic isolated congenital asplenia.

A majority of the reported adult cases of congenital asplenia were presented with thrombocytosis [2]. On the contrary, our patient had near normal number of platelets which were unusually large. To our knowledge, this is the first reported case of asplenia with giant platelets. More than 40% of the measured asplenic patient platelets diameters were larger than 4 μm, while the diameters of platelets obtained from healthy individuals ranged above that value very scarcely, around 1%. The largest measured diameter in the patient's sample (6.7 μm) was almost two folds higher than the largest value of platelets found in healthy individuals.

The unusually large platelets were noticed before in macrothrombocytopenias [14]. Although the platelets seen in our case were large size, they differed from the appearance of the macrothrombocytes seen in the macrothrombocytopenias. Ultramicrographs of the previously published cases with macrothrombocytopenias revealed aggregates with large vacuoles and areas mostly devoided of dense bodies and alpha granules [14]. In the case presented here, there were no large vacuoles seen in the platelets while both dense bodies and alpha granules were apparent.

Some reported patients with congenital asplenia had thromboembolic complications. An adult case of isolated congenital spleen agenesis complicated by thrombocytosis and chronic thromboembolic pulmonary hypertension was previously described [7] as well as the patient with congenital asplenia, thrombocytosis and myocardial infarction [5]. Our patient also had the diffuse coronary artery disease but without thrombocytosis. However, her cardiovascular condition might be related to the ultrastructurally altered platelets. It was previously shown that higher-than normal mean platelet volume may be considered as a risk factor for vascular complications [15]. It was previously noticed that the platelet size correlated with the platelet reactivity and that larger platelets had greater prothrombotic potential. The elevated platelet size (MPV) was found to be associated with increased platelet aggregation, increased expression of adhesion molecules and elevated risk of cerebro- and cardiovascular diseases [15, 16].

The number of platelets in our patient, determined by automatic counter, was significantly lower than determined immunologically by CD61 or by counting in a chamber due to the inability of automatic counter to recognize large platelets. Precise determination of platelets count in patients with giant platelets requires the use of immunological method or counting platelets in a chamber. It is particularly important when a patient has indication for anticoagulant or antiplatelet therapy, as it was in our case.

It is well-known that in the resting state the platelets are discoid whereas activated platelets develop pseudopodia or extensions from the cell wall [11]. The platelet activation is also consistent with certain morphological features such as dilatation of open canalicular system [17]. However, the open canalicular system did not show any dilatation in the cells of the specimens that we studied. Both platelets seen in the bone marrow and peripheral blood were rounded and there were no prominent extensions from the cell wall and the marginal microtubule coils could not be spotted. Perhaps the large platelets seen in our patient with asplenia were not the activated cells, but rather the cells that represented, or resembled proplatelets. Namely, proplatelets have an average diameter of 2–4 μm and can be distinguished from the platelets by their diameters, (> 2 μm vs. ≤ 2 μm. respectively) [11].

The conversion from pre- to proplatelet is driven by microtubule-based forces, which are governed by two major biophysical properties: microtubule coil diameter and microtubule coil thickness [11]. Interestingly, these forces both regulate and predict the size of circulating platelets generated by proplatelets, providing an explanation for the approximately 2 μm diameter of platelets [11]. According to the Thon and Italiano [11], circular preplatelets are released into the blood, rapidly convert into barbell proplatelets, and undergo fast rounds of abscission that result in mature platelets, or alternatively, preplatelets may become trapped in the microcapillaries of the bone marrow, lung, or spleen where intravascular shear forces drive proplatelet to platelet production. In respect to this, the absence of the spleen can lead to partial absence of transition of proplatelets to platelets.

Conclusion

The case presented in this paper is very unusual due to the presence of enlarged spherical platelets in peripheral blood and the bone marrow of the patient with congenital asplenia. Peculiarity of this case is in the presence of severe form of coronary artery disease that might be in relation with the unusually large platelets that could be in persistent activation. This unusual finding might shed the light on the role of spleen in the formation of platelets.

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Received on January 10, 2017.
Revised on September 21, 2017.
Accepted on September 22, 2017.
Online First November, 2017.

CASE REPORT

UDC: 616-053.32-007-089:617.55
https://doi.org/10.2298/VSP170322135S

Congenital diaphragmatic hernia associated with esophageal atresia, tracheoesophageal fistula and total anomalous pulmonary venous connection in a premature twin newborn

Kongenitalna dijafragmalna kila udružena sa atrezijom jednjaka, traheoezofagealnom fistulom i totalnim anomalnim utokom plućnih vena kod prevremeno rođenog blizanačkog novorođenčeta

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Abstract

Introduction. Congenital diaphragmatic hernia (CDH) with concomitant esophageal atresia (EA) and tracheo-esophageal fistula (TEF) is a very rare condition, with a high mortality rate. Prematurity and congenital heart anomalies additionally increase the mortality rate. This situation is a great challenge for clinicians, requiring multidisciplinary work and adequate treatment strategy. **Case report.** We presented a premature twin newborn at the gestational age of 33/34 weeks with body mass of 1690 g. The existence of the left CDH was established on prenatal ultrasound exam in the 24th gestational week, and the diagnosis of EA with TEF was made on admittance to our hospital. The cardiac ultrasound exam revealed the total anomalous pulmonary venous connection (TAPVC). The first operation was performed on the day of admittance and consisted of left subcostal laparotomy, diaphragmatic repair, elastic occlusion of the gastroesophageal junction and gastrostomy. The ligation of TEF and esophagoplasty were done 13 days later in the second operation. The lethal outcome during the esophagoplasty was caused by the crisis of pulmonary hypertension and associated congenital heart anomaly (TAPVC). The presence of CDH and EA/TEF in association with TAPVC in a twin newborn has not been reported before in the literature. **Conclusion.** The treatment of newborns with CDH and EA/TEF requires multidisciplinary well-coordinated team work of pediatric surgeons, anaesthesiologists, neonatologists and pulmologists. The standard protocol for the management does not exist, but well-planned staged operations could enable greater survival rate.

Key words:
infant, premature; congenital abnormalities; hernia, diaphragmatic; esophageal atresia; tracheoesophageal fistula; heart defects, congenital; digestive system surgical procedures.

Apstrakt

Uvod. Kongenitalna dijafragmalna kila (KDK) udružena sa atrezijom jednjaka (EA) i traheo-ezofagealnom fistulom (TEF) veoma je retko stanje, sa visokom stopom smrtnosti. Prematuritet i urođene srčane mane dodatno povećavaju stopu smrtnosti. Ovo stanje predstavlja veliki izazov za kliničare i zahteva multidisciplinarni rad i adekvatnu strategiju lečenja. **Prikaz bolesnika.** U radu je prikazano prevremeno rođeno novorođenče iz blizanačke trudnoće, rođeno u 33/34 gestacionoj nedelji, sa telesnom masom od 1 690 g. Postojanje leve dijafragmalne kile utvrđeno je na prenatalnom ultrazvučnom pregledu u 24. gestacionoj nedelji, dok je dijagnoza EA sa TEF postavljena po prijemu u našu bolnicu. Ultrazvučnim pregledom srca dijagnostikovano je postojanje totalnog anomalnog utoka plućnih vena (*total anomalous pulmonary venous connection* – TAPVC). Prva operacija učinjena je na dan prijema i obuhvatala je levu supkostalnu laparotomiju, rekonstrukciju dijafragme, elastično zatvaranje gastroezofagealnog spoja i gastrostomiju. Podvezivanje TEF i ezofagoplastika su učinjeni 13 dana kasnije, tokom druge operacije. Smrtni ishod tokom ezofagoplastike je bio uzrokovan krizom plućne hipertenzije i udruženom srčanom manom (TAPVC). Postojanje KDK i EA/TEF kod novorođenčeta iz blizanačke trudnoće i udruženost ovog stanja sa TAPVC do sada nisu objavljeni u literaturi. **Zaključak.**

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Lečenje novorođenčadi sa CDH i EA/TEF zahteva multidisciplinarni, dobro koordinisan timski rad dečjih hirurga, anesteziologa, neonatologa i pulmologa. Standardni protokol lečenja ne postoji, ali dobro planirane etapne operacije mogle bi da omoguće veću stopu preživljavanja.

Ključne reči:
novorođenče, prevremeno; anomalije; hernija, dijafragmalna; jednjak, atrezija; traheoezofagusna fistula; srce, kongenitalne mane; hirurgija digestivnog sistema, procedure.

Introduction

Congenital Bochdalek diaphragmatic hernia (CDH) with concomitant esophageal atresia (EA) and tracheoesophageal fistula (TEF) is a very rare entity, with extremely high mortality rate. Prematurity and congenital heart anomalies are additional conditions that increase the mortality rate. In 2004 California Birth Defects Monitoring Program, analyzing the population of 3,318.966 live births and stillbirths in the period 1983–1996, there were reported 433 cases with Bochdalek type CDH (1.3:10000 births), 893 cases with EA/TEF (2.7:10000 births), and 18 cases with CDH and EA/TEF (0.05:10000 births). The mortality rate of patients with CDH associated with EA/TEF is very high, and it was reported that 16 of 17 babies were stillborn or died soon after birth [1]. Until 2015 there were only two database publications in relevant literature, announced in 2005 and 2013, and each reports approximately 20 cases of CDH with EA from different registries [1, 2]. Only 13 cases were reported in details [2–11], and all cases were sporadic, presented as case reports. Of those 13, there were only 3 case reports with successful management and survival of an infant with left CDH, EA, and TEF [3–5]. The first single-institution series of 6 newborns with CDH, EA and TEF appeared in 2015. Five newborns were operated on, and 3 patients survived 12.

The cause of the association of CDH and EA is still unknown. Chromosomal abnormalities were reported, such as a mosaic duplication on the long arm of the chromosome 10, but the genetic cause was not identified yet [1, 5]. The reasons of the high mortality rate are pulmonary hypertension and lung hypoplasia, caused by diaphragmatic hernia, combined with gastric acid reflux into respiratory tract and gastrointestinal distension in the chest cavity, caused by TEF. Associated cardiac anomalies, such as truncus arteriosus communis (TAC) [6] and total anomalous pulmonary venous (TAPVC) in our patient, and prematurity increased further the mortality rate. The presence of EA and TEF, causing gastric acid reflux and gastrointestinal distension in the chest cavity, exclude the usually applied initial conservative treatment of CDH, based on the delay of operative treatment until pulmonary hypertension decrease. CDH with EA/TEF is a great challenge for clinicians, requiring multidisciplinary work and adequate surgical strategy.

Case report

A premature twin male infant was born in a provincial hospital by spontaneous vaginal delivery, at the gestational age of 33/34 weeks, with body weight of 1,690 g. The existence of the left diaphragmatic hernia (Bochdalek type) was diagnosed on the prenatal ultrasound exam in the 24th gestational week. No other anomalies were found. The Apgar score at birth was 1 and 2 at the 1st and 5th minute, respectively. The newborn was intubated immediately after birth, but a nasogastric tube could not be placed. Chest radiography confirmed the left sided diaphragmatic hernia. His twin brother weighed 1,900 g, with no diagnosed anomalies, and was further treated in the hospital where he was born. The newborn with diagnosed CDH and suspected EA was transferred to our hospital 6 hours after birth. At the admission, the baby was intubated, cyanotic, without nasogastric tube. He was reintubated nasotracheally and pressure-controlled mechanical ventilation was started. The attempt of placing a nasogastric tube was unsuccessful. In the neonatal intensive care unit (NICU), a small amount of barium contrast was given in the nasoesophageal tube, and the radiography confirmed the clinical suspicion of EA. The presence of air in the stomach and intestinal loops situated in the chest pointed to the presence of TEF and left sided CDH (Figure 1). The abdominal ultrasound exam showed the presence of intestinal loops in the left thoracic cavity, without anomalies on abdominal parenchimatous organs. The cardiac ultrasound exam diagnosed the patent ductus arteriosus (DAP) and pulmonary artery hypertension (HAP), and raised the suspicion of the TAPVC.

The decision for urgent operative treatment was made, and 4 hours after the admittance and the use of resuscitation measures, the baby was transferred to the operating room. Through the left subcostal incision the left hemidiaphragm was approached. There was no hernial sac. Herniated organs (stomach, small intestine, colon and spleen) were pulled out of the thoracic cavity into the operative field. The anterior rim of the left diaphragm was well-developed, and the posterior rim was present as underdeveloped. The chest tube was placed, and the primary reconstruction of the left hemidiaphragm was made by mattress sutures. Malrotation was managed by Ladd operation. Then, a rubber sling was placed around the gastroesophageal junction, pulled through the side hole of the shortened nasogastric tube, and both exteriorized through the abdominal wall, in order to perform moderate angulation and temporary occlusion of the esophagus, so preventing the disastrous effect of TEF. A classical Stamm gastrostomy was made, and the underwater testing of gastrostomy Pezzer tube showed no air leakage through the TEF in the stomach (Figure 2).

Through a period of 13 days, the mechanical ventilation and all other intensive care measures were applied, but pulmonary hypertension crisis were repeated and blood oxygenation rate varied from 32% to 82%. The chest radiography showed the recovery of the left lung. Meropenem and vankomycin were administered initially, but *Acinetobacter* was diagnosed in the tracheal aspirate, so colistimethate sodium was added [13]. Haemocultures were sterile.

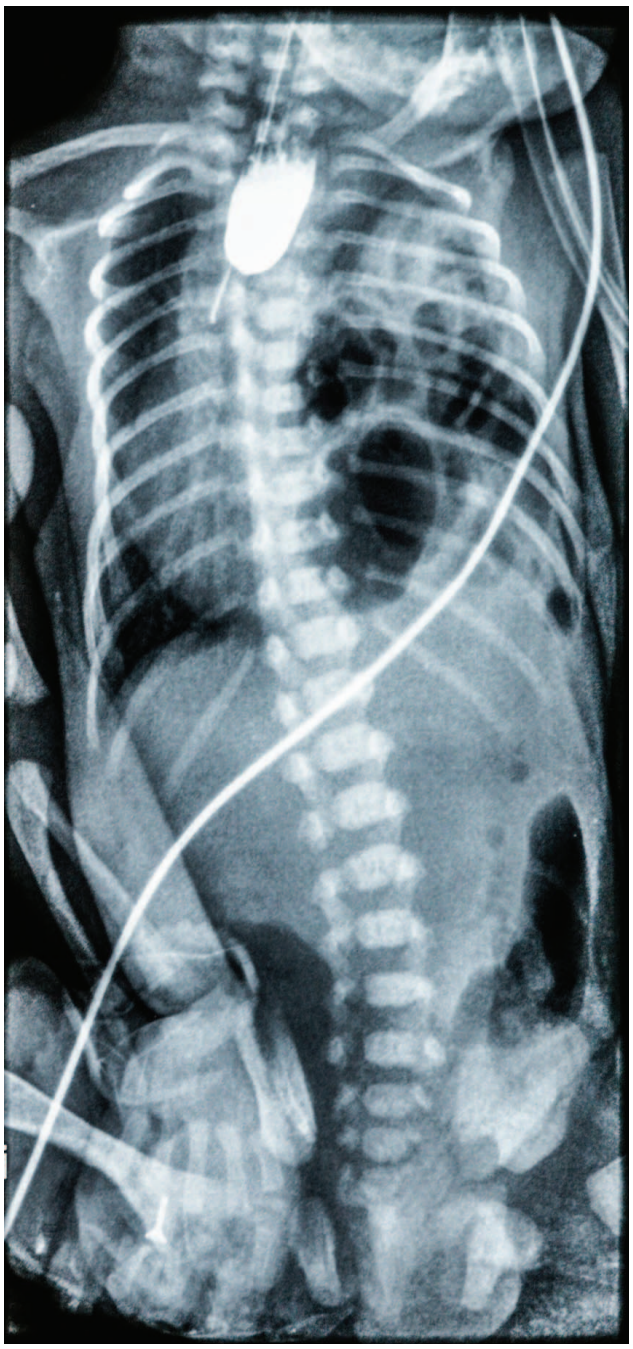

Fig. 1 – The presence of air in the stomach and intestinal loops situated in the chest pointed to the presence of the tracheo-esophageal fistula and left congenital diaphragmatic hernia.

Total parenteral nutrition was administered. Nonobstructive TAPVC was confirmed on the postoperative control cardiac ultrasound exam. The second operation was undertaken on the 13th postoperative day. Through the right posterolateral thoracotomy the TEF was approached and the large fistula was ligated and transected. The operation was continued with improved blood oxygenation rate of 80% and the esophagoplasty was started. During this procedure bradicardia, desaturation crisis, arterial hypotension and pulmonary oedema developed and, in spite of all resuscitation measures, led to the lethal outcome.

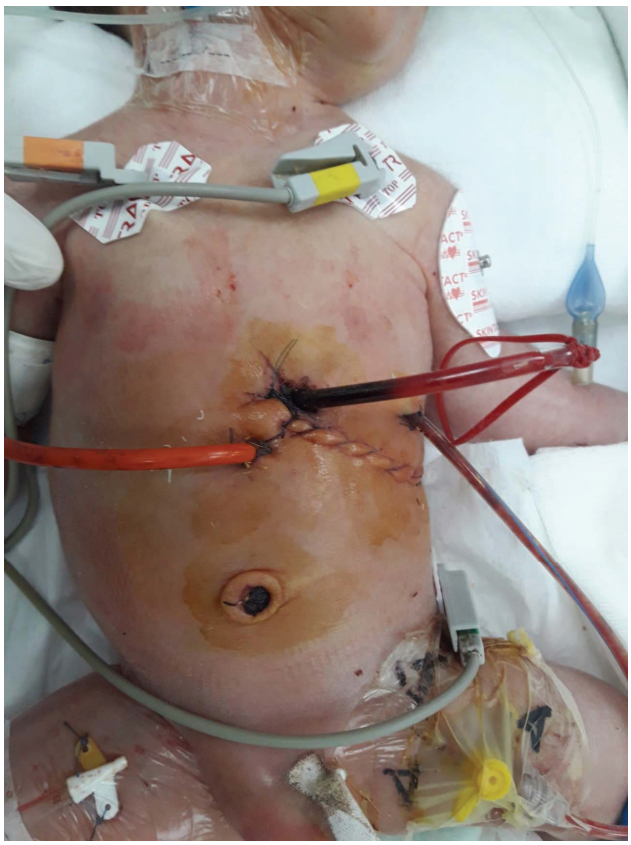

Fig. 2 – Stamm gastrostomy tested underwater showed that there was no air leakage through the Pezzer tube to the stomach.

Discussion

In babies with CDH and EA/TEF, the operative strategy is of the greatest importance. Sapin et al. [3] in 1996 reported the first successfully treated infant with CDH and EA/TEF, suggesting the laparotomic repair of CDH and avoiding thoracotomy in the first operation. The ligation of the TEF and esophagoplasty were postponed for the second operation. This attitude was based on the fact that laparotomy is better tolerated than thoracotomy, especially in the presence of pulmonary hypertension and left lung hypoplasia, which are the major problems in newborns with CDH. The disastrous effects of gastric acid reflux into respiratory system through TEF the author precluded by temporary occlusion, i.e., angulation of distal esophagus, placing a rubber sling around gastroesophageal junction. The gastrostomy was placed for gastric decompression. The ligation of TEF and esophagoplasty were performed after the baby's recovery, 2 weeks after the first operation [3]. This attitude was applied in the case reported by Charles et al. [5] in 2014, also with positive outcome and the baby's survival. An alternative strategy, the ligation of TEF and esophagoplasty in the first operation, and the repair of CDH in the second operation, with good outcome, was reported in 2013 [4]. The third operative strategy, reported by Zahn et al. [12] in 2015, suggested the CDH repair and ligation of distal esophagus 2 cm below TEF via transabdominal approach, and esophagoplasty 4–6 weeks later. This strategy was applied in 5 newborns and 3 patients survived.

In our patient with CDH, EA and TEF, we applied the principles of staged operative repair proposed by Sapin et l. [3] and Charles et al. [5]. The first operation, consisted of diaphragmatic repair, temporary occlusion of distal esophagus by silicone rubber sling and gastrostomy were performed without major problems in baby's respiratory and circulatory status, during and after the operation. So we could conclude that the proposed strategy was appropriate for babies with CDH and EA/TEF. The rubber sling around the gastroesophageal junction proved to be efficient in the temporary occlusion of distal esophagus and prevention of harmful effects of TEF. In our patient, there were no problems neither in the left hemidiaphragm repair nor in the closure of the abdominal wall, so that prosthetic material need not to be applied. The recovery of the left lung, as proved on the control postoperative chest radiography, was very satisfactory. The timing of the second operation, planned for the ligation of TEF and esophagoplasty, was determined according to literature suggestions and the baby's overall condition, and it was performed the 13th day of life. The sudden worsening of baby's condition and lethal outcome during the esophagoplasty was caused by the pulmonary hypertension crisis and associated congenital heart anomaly (TAPVC). The association of CDH with EA/TEF and TAPVC has not been reported yet in the literature. Also, the presence of CDH and associated EA/TEF in one of the twins was not mentioned in any of the references.

Conclusion

CDH (Bochdalek type) with associated EA and TEF is a very rare condition. Pulmonary hypertension and lung hypoplasia, caused by diaphragmatic hernia and gastric acid reflux into respiratory tract and gastrointestinal distension in the chest cavity, caused by TEF, lead to very high mortality rate. The treatment of these babies requires multidisciplinary, well-coordinated team work of pediatric surgeons, anaesthesiologists, neonatologists and pulmologists. The standard protocol for the management does not exist, but well-planned staged operations could improve survival rate.

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Received on March 22, 2017.
Accepted on September 25, 2017.
Online First September, 2017.

CASE REPORT

UDC: 618.177:[618.11-006:618.14-007
https://doi.org/10.2298/VSP170113148R

Uterus didelphys associated with ovarian endometriosis in an infertile patient

Dvostruka materica udružena sa endometriozom jajnika kod infertilne pacijentkinje

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Abstract

Introduction. Uterus didelphys results when Mullerian duct fusion is completely arrested during development. We presented a rare case of nonobstructive uterus didelphys occurring simultaneously with an endometriotic cyst of the ovary. **Case report.** A twenty-nine-year-old, nulliparous patient was admitted to our Clinic for laparoscopic treatment of an endometriotic ovarian cyst. Diagnoses of right ovarian endometriotic cyst and nonobstructed uterus didelphys were established with bimanual pelvic exam and two-dimensional transvaginal ultrasound. Diagnoses were subsequently confirmed by laparoscopy and magnetic resonance imaging. Laparoscopic incision and drainage of the endometriotic cyst were performed, followed by biopsy and coagulation of endometriotic lesions. Histopathology confirmed ovarian endometriosis. Gonadotropin-releasing hormone analogue (GnRHa) was prescribed postoperatively, for a total of 3 months. Ten months after completion of treatment, the patients was without disease recurence. **Conclusion.** Nonobstructive uterus didelphys is rarely associated with ovarian endometriosis.

Key words:
uterus; congenital abnormalities; ovary; infertility; endometriosis; laparoscopy; treatment outocme.

Apstrakt

Uvod. Dvostruka materica nastaje kada potpuno izostane fuzija Milerovih kanala. U radu je prikazan redak entitet dvostruke materice neopstruktivnog tipa, udružene sa endometriotičnom cistom jajnika. **Prikaz bolesnika**. Pacijentkinja stara 29 godina, nulipara, primljena je na našu kliniku za laparoskopsku operaciju endometriotične ciste jajnika. Bimanuelnim ginekološkim pregledom i transvaginalnim 2D ultrazvukom dijagnostikovana je endometriotična cista desnog jajnika i dvostruka materica neopstruktivnog tipa. Dijagnoza je kasnije potvrđena laparoskopijom i magnetnom rezonancom. Urađena je laparoskopska incizija i drenaža sadržaja ciste, sa biopsijom i koagulacijom endometriotičnog žarišta. Patohistološki je potvrđena endometrioza jajnika. Postoperativno pacijentkinji je ordiniran analog gonadotropin oslobađajućeg hormona, u vremenu od tri meseca. Deset meseci nakon završenog kompletnog tretmana, pacijentkinja je bila bez recidiva bolesti. **Zaključak.** Dvostruka materica neopstruktivnog tipa je retko udružena sa endometriozom jajnika.

Ključne reči:
materica; anomalije; jajnik; neplodnost; endometrioza; laparoskopija; lečenje, ishod.

Introduction

Mullerian duct anomalies arise as a result of duct development failure, incomplete duct fusion or canalization, or incomplete reabsorption of medial uterine septum. The prevalence of female genital tract anomalies in the general population varies from 0.5% to 5.0% [1]. Uterus didelphys arises due to complete failure of Mullerian ducts to fuse and differentiate to form uterus and cervix during the 8th gestational week. This is a lateral fusion defect of the Mullerian ducts resulting in symmetrical nonobstructive uterus didelphys accompanied by a complete longitudinal vaginal septum [2]. Most women with a nonobstructive uterus didelphys are asymptomatic, but some present with dysmenorrhea or dyspareunia in the presence of a longitudinal vaginal septum. If hemivaginal obstruction is present, symptoms tend to occur, and include hematocolpos, hematometra, hematometrocolpos as well as a lower abdominal pain [3]. Uterus didelphys, without an obstructed hemivagina, is rarely associated with endometriosis. Endometriosis is more commonly present in

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the obstructive forms of uterus didelphys. Uterus didelphys has also been described as being part of the Herlyn-Werner-Wunderlich syndrome (HWWS), also known as obstructed hemivagina and ipsilateral renal agenesis (OHVIRA syndrome) [4, 5]. Majority of cases of nonobstructive uterus didelphys do not require surgical management. Excision of vaginal septum is indicated in obstructed unilateral vagina while a hemihysterectomy is indicated in rare cases of cervical hypoplasia or agenesis [6, 7].

Case report

A twenty-nine-year-old nulliparous woman was referred to our clinic from a primary healthcare centre for laparoscopic surgery of a right ovarian endometriotic cyst. The gynecologic history revealed that menarche occurred at the age of 14 years and that menstrual cycles have been regular since. The patient has been married for four years, but has failed to become pregnant despite trying during the last 2 years. The obstetric history revealed a nulligravida. The patient denied a history of previous gynecologic disease. Upon admission to our Clinic, a complete preoperative work-up was performed, including the bimanual pelvic exam and two-dimensional transvaginal pelvic ultrasound. The bimanual pelvic exam revealed a partial longitudinal septum in the superior third of the vagina. The double cervix was 1.5 cm long, and contained openings to two endocervical canals. Two separate, firm and mobile pelvic masses were palpable, each mass the size of a small female fist. The left adnexa were mobile and non-tender to palpation. The right ovary was enlarged and tense on palpation. Two-dimensional transvaginal pelvic ultrasound revealed the following: two completely separate and equal hemiuteri. The right hemiuterus was anteverted, measuring 48 × 33 mm, characterized by normal contours and an endometrial thickness of 6 mm. The left hemiuterus was anteverted, measuring 50 × 33 mm, was characterized by normal contours and an endometrial thickness of 6 mm. A right ovarian cyst filled with viscous fluid was seen, and measured 42 × 40 mm. The left ovary measured 32 × 24 mm, and contained multiple follicles, which measured up to 6 mm. Double uterine cervix had two endocervical canals, and measured 28 mm in length. Abdominal and urinary system ultrasound revealed normal findings. Both kidneys were of normal size and morphology and were in their typical location. After preoperative work-up and preparation, the patient underwent laparoscopy. The following were the laparoscopic findings: two uteri of equal sizes were visualized in the pelvic cavity, each equivalent to the size of a small female fist. The two were completely separate, at a distance of approximately 3–4 cm from each other. Each uterus was associated with one Fallopian tube, and the Fallopian tubes were of normal length with mobile fimbria. An endometriotic cyst containing hemorrhagic debris was visualized on the right ovary and measured 40 × 40 mm. The left ovary was of normal size, morphology and whitish in colour. The laparoscopic finding is shown in Figure 1. The isthmus of the posterior uterine wall of the right hemiuterus had a subserous fibroid, which measured 1 cm. Endometriotic implants were seen on the right uterosacral ligament. As cystectomy, which would encompass complete excision of the cyst and its pseudocapsule, was technically difficult to perform, incision and drainage of the cyst was done instead, followed by biopsy and coagulation of endometriotic lesions. The subserous fibroid was excised. Both specimens were sent for the histopathological analysis. Chromopertubation, using methylene blue dye, was performed to assess tubal patency; spillage of the dye from the right tube into the abdomen indicated the patency of the right tube while the left tube filled up without spillage and was determined to be occluded at the uterine horn. Postoperative recovery was uneventful. The patient was discharged from the hospital on the second postoperative day. Pelvic magnetic resonance imaging (MRI) with paramagnetic contrast was performed postoperatively. The MRI findings confirmed a uterus didelphys, which consisted of two non-communicating uterine cavities and two cervices. A partial septum was found in the proximal third of the vagina. The MRI is shown in Figure 2.

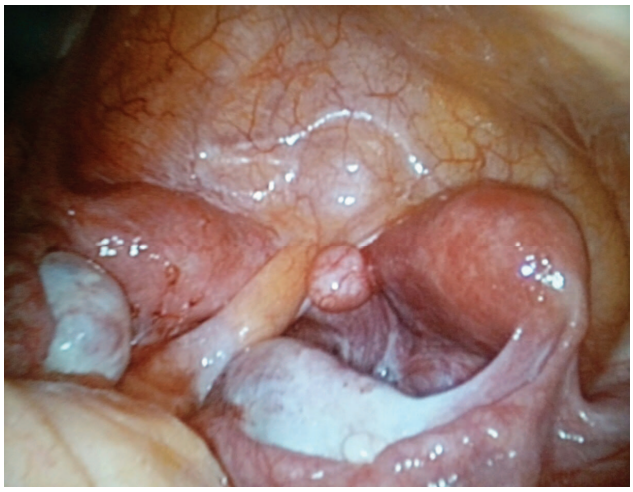

Fig. 1 – Laparoscopic findings shows two separate uterus.

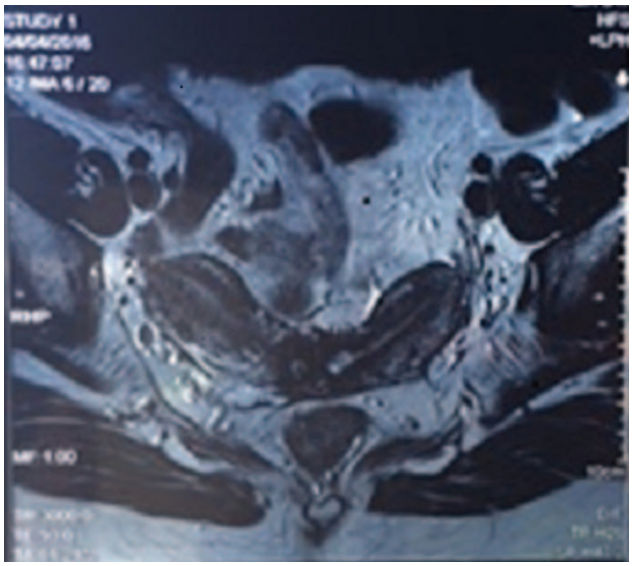

Fig. 2 – Magnetic resonance imaging (MRI) shows two separate uterus with two uterine cervix.

The final histopathological diagnosis included the following: endometriotic cyst of the right ovary; uterine fibroid. The histopathological finding is shown in Figure 3. The patient was managed with suppressive therapy using the gonadotropin-releasing hormone analogue (GnRHa), triptorelin (Diphereline® 3.75 mg), administered every 28–30 days for a total of 3 months. The patient complied with all 3 cycles of the treatment. The follow-up pelvic ultrasound and the CA-125 levels were performed and were within the normal reference range. The patient felt well and did not manifest signs of recurrent disease.

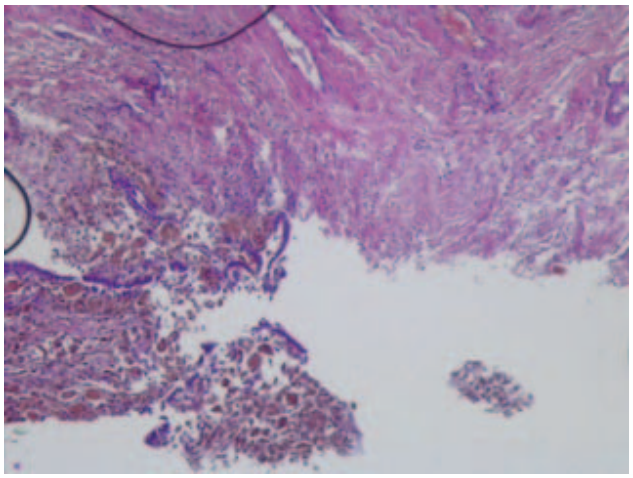

Fig. 3 – Histologic image of ovarian endometriosis (hematoxylin and eosin, ×10).

Discussion

Uterine malformations are a result of failure of Mullerian duct development, fusion, canalization or reabsorption. The prevalence of uterine anomalies in the unselected general population was 5.5%, compared to 8.0% in the population of infertile women, 13.3% in women with a history of miscarriage and 24.5% in those with miscarriage and infertility [8]. Multiple classification systems are used to classify female genital tract anomalies. The classification systems include the following: the American Society of Fertility and Sterility (AFSS); the American Society of Reproductive Medicine (ASRM); the European Society for Human Reproduction and Embryology (ESHRE) and the European Society for Gynecologic Endoscopy (ESGE) [1, 9]. Uterus didelphys constitutes 11% of all uterine malformations. Uterus didelphys arises when the Mullerian ducts completely fail to fuse leading to two separate uterine cavities and two cervices [10]. Each hemiuterus is associated with one Fallopian tube. There may be a single or double vagina. A partial or complete longitudinal septum is present in 75% of the cases [2]. Uterus didelphys was described as part of the Herlyn-Werner-Wunderlich syndrome (HWWS) [4], which is also known as obstructed hemivagina and ipsilateral renal agenesis (OHVIRA) [5, 10]. Uterus didelphys without an obstructed hemivagina is rarely associated with endometriosis. In relevant literature, there are reports of individual cases of nonobstructive uterus didelphys associated with ovarian endometriosis or peritoneal endometriosis [11]. We presented a patient with a uterus didelphys consisting of two completely separate uterine cavities and two cervices as well as a partial septum in the proximal third of the vagina, and a right ovarian endometriotic cyst. Our hypothesis is that endometriosis of the right ovary developed as a result of retrograde menstruation through the right Fallopian tube. This hypothesis is supported by the findings of chromopertubation during laparoscopy, which indicated that the right tube was patent, while the left tube was obstructed at the uterine horn. Endometriosis is more commonly present in the obstructive forms of uterus didelphys. Uterus didelphys with cervical agenesis is often associated with adenomyosis and ovarian endometriosis. Pelvic endometriosis was present in 19.15% of patients with HWWS [4]. Ipsilateral ovarian endometriosis develops almost always in cases of hemivaginal obstruction. Pelvic endometriosis is more frequent in the women with the complete hemivaginal obstruction and is found in 37% of cases, than in women with incomplete hemivaginal obstruction where it is found in 11.9% [4]. Endometriosis was found in 13.8% of women with OHVIRA, and 5.75% of these cases consisted of ovarian endometriosis [5]. The correlation between Mullerian duct anomalies and infertility is debatable. Mullerian duct anomalies occur in 3.4% of infertile women, while this prevalence increases to 4.3% in the general population, which implies that these anomalies do not negatively influence fertility [1]. Researchers reported that the women with a uterus didelphys had twin pregnancies, which developed normally within each of the uteri [12]. A term pregnancy delivered vaginally was described in the literature [13]. Most women with a nonobstructive uterus didelphys do not manifest any symptoms. If a thick longitudinal vaginal septum is present, common symptoms include dyspareunia and dysmenorrhea. Hematocolpos and hematometra arise as a result of an obstructed vaginal septum, leading to lower abdominal pain [3]. The HWWC syndrome is rare and occurs in 0.1% to 3.8% of cases. Hematocolpos and hematometra develop ipsilateral to the atretic hemivagina. This classical presentation occurs in 72.4% of HWW syndrome cases, while a rare variant of HWW syndrome occurs in 27.6% consisting of a uterine septum and cervical agenesis [14]. The diagnosis of uterus didelphys is usually established by bimanual pelvic exam, pelvic ultrasound (2D and 3D), hysterosalpingography, laparoscopy, hysteroscopy and magnetic resonance imaging [15]. The women with a nonobstructive uterus didelphys usually do not require surgical management. Excision of vaginal septum is indicated in the women with an obstructed unilateral vagina [6]. Hemihysterectomy is indicated in cases of uterus didelphys associated with unilateral cervical aplasia [7].

Conclusion

Nonobstructive uterus didelphys is rarely associated with ovarian endometriosis. Most women with a nonobstructive uterus didelphys do not manifest any symptoms. If a thick longitudinal vaginal septum is present, common symptoms include dyspareunia and dysmenorrhea. Endometriosis is more frequently associated with obstructive uterus didelphys, which manifests as hematocolpos and hematometra.

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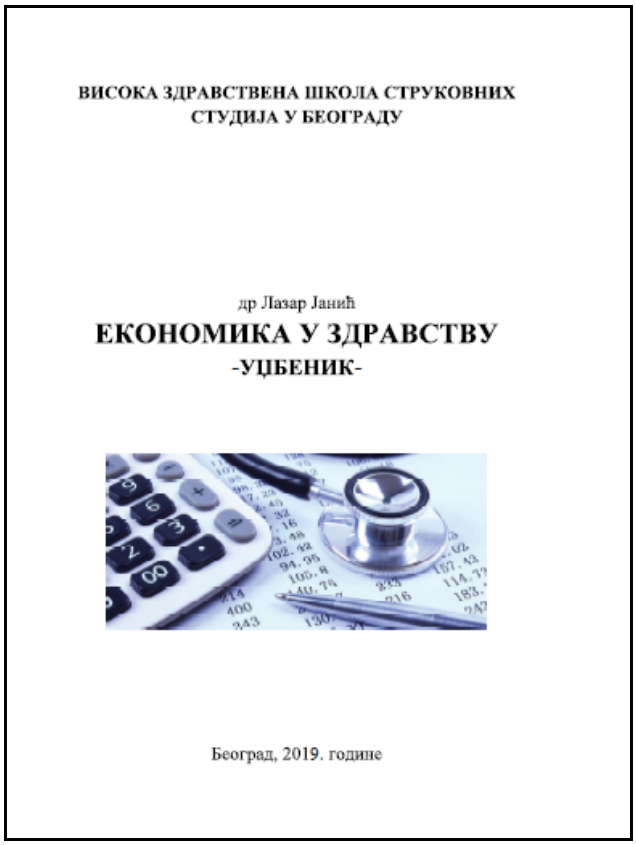
Received on January 13, 2017.
Accepted on September 28, 2017.
Online First October, 2017.

BOOK REVIEW/PRIKAZ KNJIGE

Ekonomika u zdravstvu – udžbenik



Naslov knjige: Ekonomika u zdravstvu - udžbenik

Autor: dr Lazar Janić

Izdavač: Visoka zdravstvena škola strukovnih studija u Beogradu (Zemun) i dr Lazar Janić

Mesto i godina izdanja: Beograd, 2019. godina

Štampa: Štamparija Conti d.o.o. Kneginje Ljubice br. 8, Beograd

ISBN: 978-86-920547-2-3

Knjiga „Ekonimika u zdravstvu – udžbenik" nastala je kao rezultat višegodišnjeg pedagoškog i naučnoistraživačkog rada autora. Napisana je na 272 stranice i sadrži 10 poglavlja kojima prethode predgovor i uvod, a slede zaključak i popis literature (121 referenca koja se odnosi na knjige i naučne članke na srpskom i engleskom jeziku, 23 reference koje sadrže zakone i podzakonska akta i 38 referenci iz drugih izvora).

Knjiga je usklađena sa postavkom ekonomike u zdravstvu kao naučne discipline i predstavlja kompletno, dobro strukturisano i sistematizovano delo sa nizom praktičnih primera, preporuka i smernica za efikasnu implementaciju savremenih principa upravljanja ekonomikom u zdravstvu. Knjiga je pisana akademskim stilom, bez suvišne frazeologije, prilagođenim nivou izučavanja na visokoj strukovnoj školi, odnosno pretpostavljenom nivou očekivanja i saznanja čitalaca.

Cilj ovog udžbenika je da pomogne čitaocu, pre svega studentima, u sticanju znanja iz oblasti zdravstvene ekonomike tako što predstavlja relevantne teme u okviru sistema zdravstvene zaštite kroz prizmu koncepta savremenog menadžmenta i ekonomije. Uz pomoć specifičnog pristupa i metode simplifikacije, obrađene su relevantne tematske oblasti sa predstavljanjem koncepta i principa ekonomske nauke u funkciji zdravstvene zaštite i zdravlja korišćenjem metoda i tehnika koje su svojstvene društvenim naučnim disciplinama. Čitalac, stoga, razumevajući ekonomske principe u sistemu zdravstvene zaštite jasnije sagledava zdravstveno tržište i zdravstvene politike.

Autor u prvom poglavlju uvodi čitaoce u obalast ekonomike u zdravstvu preko upoznavanja sa osnovnim pojmovima kao što su zdravlje, socijalna medicina, zdravstvena zaštita, potom prelazi na pojmovno određenje same naučne discipline i predstavljanje predmeta njenog izučavanja. U okviru drugog poglavlja predstavljeni su ekonomski aspekti zdravlja i zdravstvene zaštite, ukazano je na neizvesnost zdravstvenih potreba i asimetričnu informisanost, kao i na pozitivne i negaitvne eksterne efekte u zdravstvu. Potom je, po uzoru na druge ekonomske discipline, analizirana i uloga države u ovim procesima. U trećem poglavlju autor kroz istorijski kontekst predstavlja zdravstveno osiguranje i odnosne postulate. Kroz četvrto poglavlje autor prikazuje alokaciju resursa koji su namenjeni zdravlju stanovništva, te ukazuje na deficit istog. U okviru petog poglavlja uočavaju se anomalije zdravstvenog tržišta nakon što se definišu njegovi osnovni elementi: ponuda i tražnja zdravstvenih usluga. Autor u okviru šestog poglavlja predstavlja modele finansiranja u zdravstvu, ukazujući i na potrebu za reforomom finansiranja zdravstva u Repubici Srbiji. Kroz sedmo poglavlje predstavljena je oblast farmakoekonomike, odnos ponude i traženje, s akcentom na potencijalne i postojeće koflikte i teškoće, kritičnu ulogu marketinga i promocije. U okviru osmog poglavlja autor preispituje etičnost u zdravstvu i koncept „dostignute potrebe“, dok se u devetom poglavlju kompariraju modeli različitih nacionalnih zdravstvenih sistema, te u desetom predstavljaju izvori finansiranja zdravstvene zaštite u Srbiji i zdravstvena potrošnja (obim i struktura iste).

Teme koje je autor odabrao za teorijsku i stručnu analizu su aktuelne, ako se u vidu imaju uslovi u kojima funkcionišu zdravstvene institucije u okruženju i u okolnostima kada je smanjenje troškova u fokusu, specifičan odnos ponude i tražnje, postojanje moralnog hazarda i asimetričnosti u informisanju. Intencija dr Janića predstavlja realan pristup problematici zasnovan na poznavanju relevantne formalne literature iz oblasti ekonomije i ekonomike u zdravstvu, ali i poznavanja nacionalne i međunarodne regulative i prakse.

Svojim konceptom, metodologijom i sadržajem knjiga dr Lazara Janića predstavlja značajan doprinos implementaciji ekonomskih i etičkih principa u zdravstvu, imajući u vidu postojanje naučnoistraživačkog deficita u ovoj oblasti na nacionalnom i međunarodnom nivou, zbog čega se može slobodno reći da ona predstavlja i svojevrstan naučni doprinos. Njen društveni doprinos ogleda se u mogućnosti izgradnje platforme za bolju implementaciju naučenog u principe poslovanja zdravstvenih insitucija na svim nivoima, i u buduću nacionalnu regulativu.

Koncipirana kao udžbenik namenjen, pre svega, studentima Visoke zdravstvene škole strukovnih studija u Beogradu, ova publikacija, sigurno, može biti korisna kao osnovna ili dopunska literatura i studentima drugih akademskim institucija koji u okviru nastavnog programa imaju zastupljene sadržaje iz ekonomike u zdravstvu, ali i stručnjacima iz ove oblasti. Naime, materija koja je u knjizi izložena i ponuđeni opseg prezentovanih primera iz prakse, deo je napora autora da pruži mogućnost i onima koji se profesionalno bave ovom disciplinom da nadograde svoja znanja u domenu primene savremenih rešenja iz oblasti ekonomike u zdravstvu u svojim institucijama.

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

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

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

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

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

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