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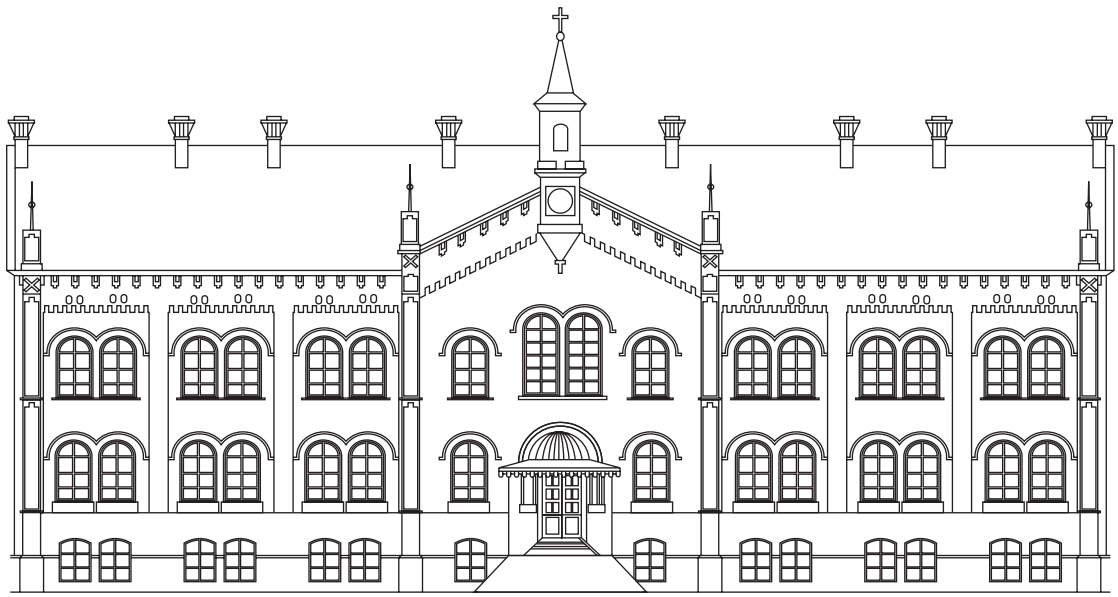

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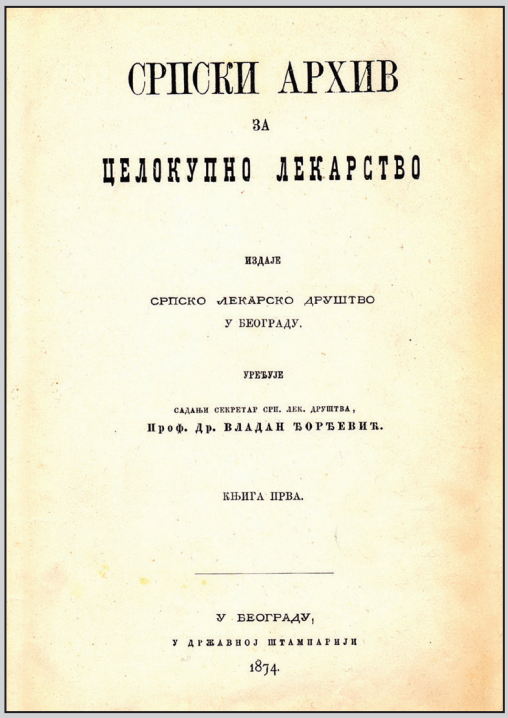

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ЗА

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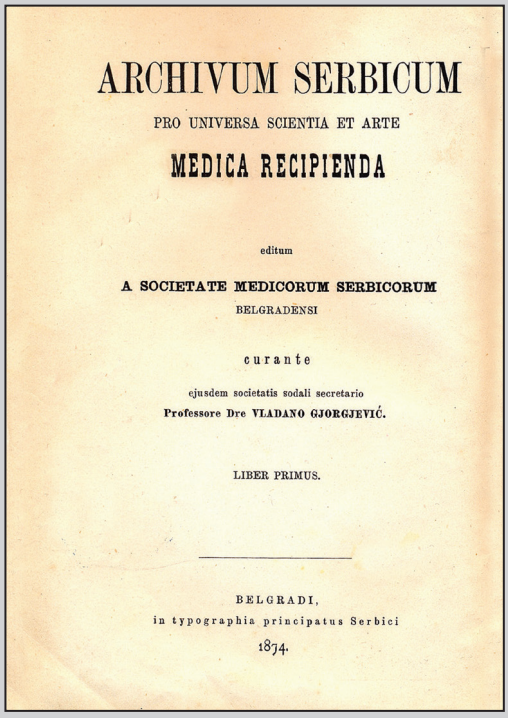
У БЕОГРАДУ,

У ДРЖАВНОЈ ШТАМПАРИЈИ

1874.

Прва страна првог броја часописа на српском језику



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1874.

The title page of the first journal volume in Latin

Српски архив за целокупно лекарство је часопис Српског лекарског друштва основаног 1872. године, први пут штампан 1874. године, у којем се објављују радови чланова Српског лекарског друштва, претплатника часописа и чланова других друштава медицинских и сродних струка. Објављују се: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике и регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози.

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Српско лекарско друштво
Џорџа Вашингтона 19, 11000 Београд, Србија
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Српски архив
Краљице Наталије 1, 11000 Београд, Србија
Телефон: +381 (0)11 409 27 76
+381 (0)11 409 44 79
Е-пошта: office@srpskiarhiv.rs
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ПРЕТПЛАТА И ЕКСПЕДИЦИЈА
Српско лекарско друштво
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Телефон: +381(0)11 3245-149
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EDITORIAL OFFICE
Serbian Archives of Medicine
Kraljice Natalije 1, 11000 Belgrade, Serbia
Phone: +381 (0)11 409 27 76
+381 (0)11 409 44 79
E-mail: office@srpskiarhiv.rs
Website: www.srpskiarhiv.rs

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Impact of the COVID-19 pandemic on dental practice in Serbia – prospective study

Filip Ivanjac, Vitomir Konstantinović, Biljana Miličić
University of Belgrade, School of Dental Medicine, Belgrade, Serbia

SUMMARY
Introduction/Objective The COVID-19, pandemic had a great impact on all spheres of dental practice. Dentists are the most affected category, due to their line of work. Studies conducted worldwide have shown a range of repercussions in dentistry including lockdowns, limited access to dental services, changes in prices, working hours and availability of protective equipment, increased anxiety levels, changes in the protocols, and personnel fear of contracting the disease at work.
The aim of this prospective observational survey study was to evaluate the impact of the COVID-19 pandemic on the dental practices in Serbia, as well as the challenges and consequences faced by dentists since the beginning of the pandemic, via an anonymous questionnaire.
Methods Multi layered questioner was used divided in to four sections: 1. Demographic; 2. Dental office professional experience; 3. Epidemiological professional experience; 4. Personal pandemic experience.
Results In total, 459 members of the Serbian Dental Chamber participated, gender distribution was 34.4% men and 65.6% women, age range was 26–81 years, of which 76.4% were immunized against COVID-19. Professional, epidemiological and personal experience showed high level of preventive measures, overcoming professional limitations in order to lower the probability of contracting and spreading the disease.
Conclusion The COVID-19 pandemic had a large influence on the dental practice in Serbia. Many dentists had to overcome the professional, economic and personal limits. The immunization made all the difference and created a safer environment for dentists and patients.
Keywords: pandemic; COVID-19; dental practice

INTRODUCTION

The outbreak of the COVID-19 pandemic in December 2019 caught the worldwide healthcare providers unprepared. The epidemiological situation in Serbia in the early 2020 was similar. Medical practitioners did not know how to cope with the pandemic, since there was no conventional therapy or immunization. The only means of prevention was the protective equipment, which was not always available. The scientific data and the epidemiologist recommendations were essential [1, 2, 3].

However, the COVID-19 pandemic had a great impact on all spheres of healthcare, one of which was dental practice. Many studies worldwide have shown that dentists were one of the most affected categories, due to their line of work. Dental medicine doctors were at the first line of health risk since they work face to face with patients. The purpose of this study was to collect data in order to help dentists to cope better with future epidemiological risks [4, 5, 6].

A highly contagious severe acute respiratory syndrome coronavirus 2 (SARS-CoV2), is easily transmitted during dental procedures that commonly generate blood and saliva aerosols that could lead to the infection. The instruments such as turbine and cavitron generate aerosols, the mist formed of micro droplets of saliva and/or blood that float in the air creating a potentially contagious environment [7, 8, 9].

Since the infection rates were high and the resources limited, many dental practitioners had to close their offices temporarily, change protocols, increase protection and change prices. All of that influenced the dentists from the socio-economic, professional and psychological point of view. Likewise, the studies conducted worldwide showed a range of consequences in dentistry that included lockdowns, limited access to dental services, changes in prices, working hours and availability of the personal protective equipment, increased anxiety levels, changes in dental protocols and personnel's fear of contracting the disease at work [7, 8, 9]. Many dental offices in our country reported significant changes in the number of patients per month before and during the pandemic. They caused changes in income, working hours and standard treatments. The Ministry of Health and the Serbian Dental Chamber gave recommendations on how to change protocols, increase the protection of patients and dental practitioners, and how to organize dental practice work in a safe manner. The question is how many dental practitioners followed these instructions since they were not mandatory. With the availability of vaccine and the strong anti-vaccine propaganda on the other side, one of the questions is what percentage of dental

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Correspondence to:
Filip IVANJAC
Svetogorska 18
Belgrade 11000
Serbia
filipivanjac@yahoo.com

practitioners was immunized. Dental tourism is a significant source of patients for many dental offices, so one of the questions is if the dentists asked foreign citizens for a valid pandemic-related documentation.

The aim of this prospective observational survey study was to evaluate the impact of the COVID-19 pandemic on the dental practices in Serbia, as well as the challenges and consequences faced by dentists since the beginning of the pandemic, via an anonymous questionnaire.

METHODS

The structured anonymous questionnaire was distributed to the members of the Serbian Dental Chamber via the e-mail database. The multi-layered questionnaire was divided into four sections:

1. Demographic;
2. Dental office professional experience;
3. Epidemiological professional experience;
4. Personal pandemic experience.

The anonymous questionnaire was created and filled out with Google services. All the data was sorted in an Excel base, and then the SPSS statistical program (SPSS Inc., Chicago, IL, USA) was used for further data analysis. The data was statistically analyzed and the variables were cross-referenced. The questionnaire was oriented towards the Serbian private and public dental healthcare sector with a specific regional center orientation: Belgrade, Novi Sad, Niš, Kragujevac (as major cities), Belgrade region, Central Serbia, Vojvodina, Southern Serbia, Eastern Serbia, Western Serbia, Kosovo and Metohija. In consideration to the level of education and the field of dentistry, there were general dentists, specialists, PhD/magister, primarius, oral surgeons, dental prosthetists, orthodontists, conservative dentists, periodontist, pediatric dentists.

The study was approved by the Ethical committee of the School of Dental Medicine, University of Belgrade, no. 36/29.

RESULTS

The demographic data gave us an insight into the participant structure: a total of 459 members of the Serbian Dental Chamber answered the questions anonymously. The participant gender distribution was 34.4% men and 65.6% women, whose age range was 26–81 years with the highest frequency among the dentists 39–40 years old. A majority of the participants were general dentists 70.7%, the other 29.3% were distributed among different specializations. A majority of them were without any post-graduate levels, 60.8%, specialists 29.8%, PhD 8.3%, primarius 1.1%. The private sector employees participated with 74.3%, while there were 25.7% from the public sector (Tables 1 and 2).

Table 2. Regional participant distribution

Regional participant distribution	
Belgrade	37.4%
Novi Sad	8.3%
Niš	5.3%
Kragujevac	2.4%
Belgrade County	2.4%
Vojvodina	13.3%
Western Serbia	7.2%
Eastern Serbia	4.4%
Southern Serbia	7.2%
Central Serbia	10.7%
Kosovo and Metohija	1.3%

The part of the questionnaire titled "Professional experience in dental practice" gave us an insight into the modified approach to a patient in the pandemic circumstances: a majority (84.9%) of all the participants triaged their patients during the first visit and 73.3% had a questionnaire about the pandemic, 65.1% asked if their patients were immunized, and only 8.8% asked for the immunization certificate. Most of them (72%) asked if their patients had travelled to high-risk regions, and 76.8% of the dentists inquired if they had had risky contacts recently. More than a half of the participants (52.1%) measured the patients' temperature before the treatment, 88.8% asked if their patients had flu-like symptoms, and 91.2% postponed the intervention if their patients had any flu-like symptoms. Only 10.1% asked for a valid negative COVID test and 1.5% refused to treat non-immunized patients.

In the personal history anamnesis, 79.8% of the dentists asked if their patients had previously had COVID infection, and 55.1% of dentists stated that their patients

Table 1. Participant structure

Male	159/34.4%							
Female	300/65.6%							
Total	459/100%							
Age	Range 26–81	Highest frequency 39–40						
Sector	Public 25.7%	Private 74.3%						
Years of practice	< 10 30.4%	> 10 33%	> 20 16.8%	> 30 15.1%	> 40 4.6%			
Post-graduate level	None 60.8%	Spec. 29.8%	PhD 8.3%	Prim. 1.1%				
Field of dentistry	General 70.7%	Oral surgery 6.3%	Prosthetics 5.5%	Conservative 7.7%	Orthodontics 6.8%	Periodontology 1.8%	Maxillary surgery 1.3%	Pediatric dentistry 0%

had had post-COVID consequences. They emphasized cardiologic issues as dominant consequences in 39.2%, fatigue was present in 27.8%, multi-system consequences were dominant for 21.9%, and respiratory illnesses in 11%.

Nearly one third of the dental offices in Serbia that participated in this query 31.3% had attended to foreign citizens 84.4% of dentists asked for green certificate or negative test.

The part of the questionnaire referring to "Professional epidemiological experience" gave us an insight into the pandemic influence on dental offices' business, modified protocols and risk assessment.

According to the participants, an average number of monthly patients in a dental office before the pandemic was 50–100. During the pandemic, this number decreased to an average of less than 50 patients per month. Also, during the pandemic, 64.6% dentists had to close their dental offices temporarily, and 51.9% shortened their working hours. Majority 89.7% of the participants noticed a decrease in the patients' visits' frequency, and 81.6% said the pandemic had decreased their amount of work. The prices did not change in 67.9% of dental offices. When asked if they had felt safe while working in dental offices, 50.9% of the dentists stated that they had felt endangered at their workplace, and 68.6% were afraid of exposing their families to infection. In general, 77.7% reported that the patients had asked them more for dental advices by phone. When asked about the following the updates on the epidemiological situation, 65.3% of the dentists reported that they had followed new scientific information of the pandemic regularly, 26.4% followed them from time to time and 8.4% did not follow them at all.

As far as the risk at the workplace is concerned, 64.6% of the participants evaluated their dental office as high-risk. Over 96.5% increased their level of personal protection. The protective equipment included mask (99.3%), gloves (98.9%), and face shield visor (85.1%), as indispensable, while protective goggles (58%) and disposable paper suits (48.1%) were less in use. The majority of the participants used epidemiological masks KN95 (73.2%), followed by surgical masks (42.7%), cotton masks (7.5%), while 21.9% combined two masks at once.

The recommendations of the Ministry of Health and the Serbian Dental Chamber were followed by 83.2% of the dentists. A majority (66.2%) of the dentists stated that they had avoided the use of the instruments that generate aerosols, such as turbine and cavitron, and 38.2% said they had rinsed the patients' mouths with hydrogen peroxide and povidone-iodine solution in order to prevent the infection spreading. However, 97.1% disinfected the workplace between the patients, 85.5% changed the protocols in their offices, 89% had longer intervals between the patients, 90.1% received the patients by the level of urgency, and 60.1% tried to do the treatments in fewer sessions.

We report on the dentists' personal experience during the pandemic. The dentists in Serbia got immunized against COVID-19 in 76.4% of the cases (which leaves 23.6% of non-immunized dentists). A majority of them (67.6%) have received three doses so far, and 28.8% have received only two doses, while the fourth dose has been received only by 2.9%. When it comes to the most applied vaccine among the participants, Pfizer with 46.6% and Sinopharm with 33.5% were the brands that instilled most confidence among the dentists. On the other hand, Sputnik (7.2%), Astra Zeneca (1.1%), and Moderna (0.4%) were not so popular among the dentists. The combination of two or more vaccines was received by 10.8% of the dentists (Table 3).

Table 3. Immunization parameters

Immunization parameters					
Immunized	76.4%				
Non-immunized	23.6%				
Number of doses	1 = 0.3%	2 = 28.8%	3 = 67.6%	4 = 2.9%	5 = 0.3%
Pfizer	46.6%				
Sinopharm	33.5%				
Sputnik	7.2%				
Moderna	0.4%				
Astra Zeneca	1.1%				
Sinovac	0.4%				
Johnson & Johnson	0%				
Combination	10.8%				

The data on the immunization was cross-referenced with the major cities and regions in Serbia and with the education level. We concluded that the dentists in Belgrade, with 77.8%, and in the major cities like Novi Sad, with 73.7%, and Kragujevac, with 81.8%, were the most immunized; however, Niš is one of the major cities with slightly lower immunization rate (66.7%). When it comes to the regional distribution, greater Belgrade region and Vojvodina had the highest immunization rates, 72.7% and 86.9%, respectively, while Kosovo and Metohija region was among the least immunized parts of the country with 33.3%. In relation to the post-graduate level, the immunization was mostly conducted among the dentists with a higher level of education, PhD and specialists were immunized in the percentage of 84.2% and 82.4%, respectively. However, 80% of primarius doctors were immunized, while general dentists were slightly less immunized (72.3%) (Table 4, Figure 1).

Table 4. Regional distribution of dental healthcare workers immunization

City/region	Percentage of immunized dentists
Belgrade	77.8%
Novi Sad	73.7%
Niš	66.7%
Kragujevac	81.8%
Belgrade region	72.7%
Vojvodina	86.9%
Western Serbia	69.7%
Eastern Serbia	75%
Southern Serbia	75.8%
Central Serbia	75.5%
Kosovo and Metohija	33.3%

When it comes to the COVID testing, 82.9% of the dentists were tested, of which 21.8% were tested only once,

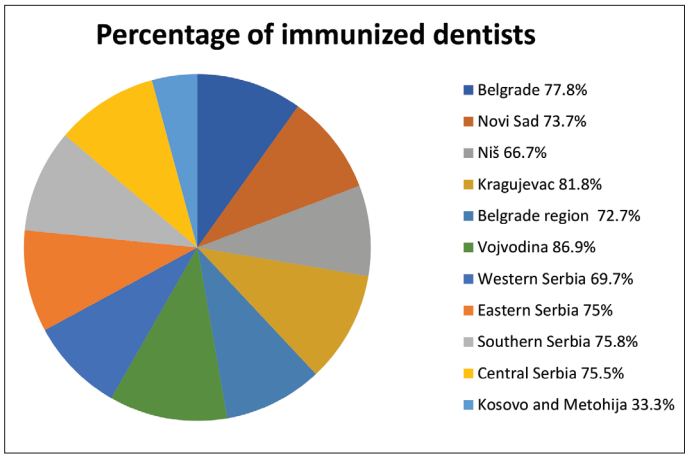

Figure 1. Percentage of immunized dentists

Table 5. Screening for COVID-19

Screening	
Tested	82.9%
Non-tested	17.1%
Tested 1	21.8%
Tested 2	27.7%
Tested 3	22.8%
Tested 4	6.2%
Tested 5	9.4%
Tested 10	4.3%
Tested > 10	1.6%

Table 6. Epidemiological parameters

Epidemiological parameters	
Had COVID	68.6%
Did not have COVID	31.4%
COVID positive once	67.7%
COVID positive twice	24.9%
COVID positive three times	6.7%
COVID positive four times	0.6%
Mild symptoms	50.9%
Moderate symptoms	42.5%
Severe symptoms	6.6%
Post-COVID consequences	26.4%
No consequences	73.6%
Mild consequences	44.3%
Moderate consequences	47.4%
Severe consequences	8.2%
Fatigue	28.3%
Cardiologic	29.3%
Respiratory	12%
Multi-system	30.4%

27.7% twice, and 22.8% three times. Only 4.3% were tested 10 times. However, when it comes to the contraction of the disease, 68.6% of dentists contracted COVID, of which 67.7% were infected only once, 24.9% twice, and only 6.7% were COVID positive three times (Tables 5 and 6).

As far as the clinical picture is concerned, half (50.9%) reported mild symptoms, while 42.5% had moderate, and 6.6% had severe symptoms. Post-COVID consequences were present in 26.4%, of which 44.3% had mild, 47.4% had moderate, and 8.2% had severe consequences. Among the dentists who participated reported on post-COVID symptoms such as fatigue 28.3% as one of the dominant manifestations, 29.3% had cardiologic, 12% respiratory, and 30.4% had multi-system issues (Table 6).

Finally, when asked about the professional risk level, the participants estimated the health risk in dentistry as: high (41.9%), moderate (32.1%), and low (26%).

DISCUSSION

The global COVID-19 pandemic influenced all spheres of healthcare [10]. To what extent it had affected dentists and their practice in Serbia was a logical question that needed to be answered, in order to help the professionals better face the future epidemiological threats.

In order to help the professionals face the future similar situations and draw conclusions from this pandemic, we designed this prospective consultative study based on the anonymous structured questionnaire. The response of the dentists was satisfactory, women were more involved in this study since they made nearly two thirds of the participants (65.6%). The age of the participants varied from 26 to 81 with the majority aged 39–40 years old. This means that a wide span of dentists was interested in this topic and that professionally most active individuals were among the ones that engaged the most in this study. A majority of the participants belong to private sector general dentists, so we assume that, as a majority of dentists in Serbia work in private sector, a majority of them are general dentists, however they were most exposed to the pandemic and had to modify protocols on their own based on their business strategy. They were the ones who felt the influence of the pandemic in all aspects. Some of them had to temporarily close their dental offices or at least to shorten their working hours. That is just one of the reasons that affected their socio-economical aspects. A majority of them noticed a decrease in the patients' visits, the amount of work and the average number of patients per month, however many of them did not change prices. Many of them made longer intervals between the patients, so they could not treat as many patients daily as usual. The result of taking all this into account was a lower income of the private dental sector. Nevertheless, their expenses increased, having in mind the increase of prices of the dental materials, protective equipment and sanitary materials.

The pandemic found many health professionals all over the world unprepared, nevertheless the majority of the dentists in our country were resourceful [11]. They triaged the patients, had a questionnaire about the pandemic, asked if their patients had been immunized, they often asked for the immunization certificate and some of them even refused to treat non-immunized patients. Nearly half of the dentists measured the patients' temperature, asked if they had flu-like symptoms and postponed the intervention if they had. Some asked for a negative COVID test. This suggests that a significant percentage of the dentists

took all the precautions to work in a COVID-free environment, to protect their patients and themselves.

Some of the questions asked by the dentists regarded the patients' health at the time. More than a half of the dentists stated that their patients had had post-COVID consequences. A majority of them listed cardiovascular problems, fatigue, multi-system consequences, respiratory issues. This was of great importance because the dentists had to modify the therapy and the treatment in order not to compromise the patients' already impaired health [12].

The dentists mostly followed the instructions of the Ministry of Health and the Serbian Dental Chamber. They scheduled the patients with longer intervals between them, received the patients by the level of urgency, and also rinsed the patients' mouths with hydrogen-peroxide and povidone-iodine solution prior to the intervention, in order to lower the probability of generating contagious aerosols; many of them avoided using Cavitron and turbine or other instruments that generate aerosols, and disinfected their workplace between the patients.

Most of the dentists were aware of the threat and they increased the level of personal protection. They stated that they had felt endangered at their workplace and evaluated their offices as high-risk, a majority of them used personal protection equipment such as masks, gloves, and face shield visors, and some of them used protective goggles and disposable paper suits. Most of them used epidemiological KN95 masks, surgical, and cotton masks, and some even used a combination of two masks at once. They followed the new scientific data and tried to be informed about the situation at the time. On top of that, they were afraid of exposing their families to the infection. This suggests that dental professionals did most of what was available to protect themselves and the patients, still not knowing entirely in what ways the disease was transmitted. Subsequently, they tried to protect their families by not exposing themselves to the infection by lowering the risk level in the dental offices [12, 13].

Since the immunization is one of the pillars of the modern medicine, one of the main questions was what percentage of dentists in Serbia was immunized. In spite of strong anti-vaccine propaganda, we saw that this percentage was relatively high, but also that it could be much higher: 76.4% immunized, 23.6% non-immunized. By cross-referencing the data, we concluded that the immunized dentists were more committed to the protection of their patients, followed the preventive instructions and made a safer environment for the patients and themselves. A majority of the dentists were immunized with three doses received, nearly one-third received only two doses, and just a small percentage received the fourth dose (Table 3). This indicates that a majority of the dentists were cautious in the beginning of the vaccination in Serbia, many of them contracted infection in the meantime (before or after the vaccination) and did not find it necessary to continue the immunization process. Since the immunity to the infection lasts for a limited period of time, a continuous immunization is necessary and the dentists in Serbia should be encouraged via the positive propaganda towards the vaccination. Pfizer and Sinopharm were the brands that instilled confidence in most participants. Other brands did not arouse that much interest among dentists. The interest in certain vaccine brands depended also on their availability, so there are dentists who received a combination of two or more vaccines. In addition, the dentists who were immunized were the ones in big cities, more prosperous regions, and with a higher level of education. This suggests that the dentist in the capital and the major cities were the ones who were more informed of the pandemic risks and the immunization benefits in this particular situation, however the dentists with a higher level of education had an easier access to the scientific information and had a better knowledge of how to protect themselves and their patients.

Most of the dentists in our study were tested for COVID-19 up to three times, however there was a small percentage of those who had been tested up to 10 times. A majority of the dentists who were positive had COVID up to three times, and they stated that their symptoms had been mild to moderate, and some of them had severe symptoms. However, mild to moderate post-COVID consequences were often reported, and some had severe consequences. Cardiologic consequences were dominant, and multi-system issues, fatigue and respiratory symptoms, were often noted (Tables 5 and 6). This indicates that COVID-19 is a disease which can be professionally limiting, leaving the consequences that can be hardly treated. The pandemic influenced dentists in such way that many of them could not work for some period of time even after the recovery from the disease because of the long-lasting post-COVID consequences. Some dentists have not been fully functional professionally to this day [14].

CONCLUSION

The COVID-19 pandemic had a large influence on the dental practice in Serbia. Many dentists had to overcome the professional, economic and personal limits. The immunization made all the difference in the dental practice and created a safer environment for dentists and patients.

Conflict of interest: None declared.

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Утицај пандемије ковида 19 на стоматолошку праксу у Србији – проспективна студија

Филип Ивањац, Витомир Константиновић, Биљана Миличић

Универзитет у Београду, Стоматолошки факултет, Београд, Србија

САЖЕТАК

Увод/Циљ Пандемија ковида 19 имала је велики утицај на све сфере стоматолошке праксе. Стоматолози су најугрожења категорија, због природе свог посла. Студије спроведене широм света показале су низ последица у стоматологији које укључују затварање ординација, ограничен приступ стоматолошким услугама, промене у ценама, радном времену и доступности заштитне опреме, повећан ниво анксиозности, промене у протоколима и страх особља од заразе на послу.

Циљ ове проспективне опсервационе анкете био је да се путем анонимног упитника процене утицај пандемије ковида 19 на стоматолошку праксу у Србији и изазови и последице са којима се стоматолози суочавају од почетка пандемије.

Методе Коришћен је вишеслојни упитник, који је подељен у четири секције: 1. демографски подаци; 2. професионално искуство у стоматолошкој ординацији; 3. епидемиолошко професионално искуство; 4. лично искуство током пандемије.

Резултати Учествовало је 459 чланова Стоматолошке коморе Србије. Било је 34,4% мушкараца и 65,6% жена, узраста 26–81 године, од чега је 76,4% вакцинисано против ковида 19. Професионално, епидемиолошко и лично искуство показало је висок степен превентивних мера и превазилажења професионалних ограничења у циљу смањења вероватноће заразе и ширења болести.

Закључак Пандемија ковида 19 имала је велики утицај на стоматолошку праксу у Србији. Многи стоматолози морали су да превазиђу професионална, економска и лична ограничења. Имунизација је направила разлику и створила безбедније окружење за стоматологе и пацијенте.

Кључне речи: пандемија; ковид 19; стоматолошка пракса

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Assessment of blood transfusion use during hospital treatment of COVID-19 patients – a single center experience

Vojislava Milutinović[1], Mirjana Kovač[2,3], Bogdan Crnokrak[1,2], Marija Zdravković[1,2]

[1]Bežanijska Kosa University Hospital Medical Center, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[3]Blood Transfusion Institute of Serbia, Belgrade, Serbia

SUMMARY

Introduction/Objective There is very limited data regarding the need for transfusion and its effect on the overall mortality of patients with coronavirus disease 2019 (COVID-19). The aim of our study is to determine the need for blood component transfusion in patents treated for COVID-19 infection.

Methods This retrospective observational study included 4426 COVID-19-positive patients treated at the Bežanijska Kosa University Hospital Medical Center between June 23, 2020 and May 2, 2021. Of these patients, 826 were treated in the intensive care units of the hospital. Of the total number of patients, 326 (7.4%) received transfusions. The clinical presentation, the structure of the applied transfusion therapy, the laboratory parameters, and the treatment outcome were analyzed in this study.

Results Of the 828 patients treated in the intensive care units, 151 (18.2%) patients required transfusion, while transfusion was necessary in a total of 4.9% of patients treated in the hospital wards. Of the total number of all transfused patients, 86% received erythrocytes, one-third of them received fresh frozen plasma, 10% received cryoprecipitate, while platelets were administered in around 6% of the patients. The mortality rate in the tested group was 46%.

Conclusion The frequency of the application of blood components was significantly higher in patients with a severe form of the disease. The presence of comorbidities did not affect the need for transfusion therapy. In the group of patients treated in the intensive care units, 85% received erythrocytes, 39% received fresh frozen plasma, 19% received cryoprecipitate and 7% received platelets.

Keywords: transfusion; blood components; COVID-19

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly contagious and pathogenic coronavirus which emerged in late 2019, causing the pandemic of the acute respiratory disease – COVID-19 [1]. This disease caused the greatest global health crisis since the influenza pandemic of 1918 and led to the death of more than 6.5 million people worldwide [2].

COVID-19 is a respiratory and vascular disease which also significantly affects the hematological system and the hemostatic system. It is associated with hypercoagulability, which is directly induced by virally mediated damage or injury to the vascular endothelium, which is caused by cytokines [3].

Clinical presentation varies – from asymptomatic forms of the disease to respiratory insufficiency, which requires mechanical ventilation. Between 17.9% and 33.3% of patients have the asymptomatic form of the disease [4, 5].

In patients with the severe form of COVID-19, acute respiratory distress syndrome may develop, usually a week after the onset of symptoms [6]. Approximately 23% of patients develop the severe form of the disease with a mortality rate of around 6% of COVID-19 patients [7].

There is little data on the need for transfusion and its influence on the overall mortality of patients with COVID-19. Several publications have shown that, in total, 6.2–13.4% of all patients with the COVID-19 infection require transfusion support [8, 9, 10].

According to the data from the few studies available, the application of blood products is low in mild to moderately severe forms of the disease, and higher in patients with severe clinical presentation [11, 12]. Of the total number of all the components administered, 83% are erythrocytes [12].

The aim of this study is to present the need for transfusion of blood components in our patients treated for COVID-19, the clinical characteristics of these patients, the composition of the transfusion therapy administered, as well as the treatment outcome.

METHODS

The research was carried out as a retrospective observational study, which included 4426 COVID-19-positive patients, treated at the Bežanijska Kosa University Hospital Medical Center, in the period between June 23, 2020 and May 2, 2021, of whom 826 were treated in the

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Correspondence to:
Vojislava MILUTINOVIĆ
Bežanijska Kosa University Hospital Medical Center
Dr Žorža Matea bb
11070 Belgrade, Serbia
vojislavasladic@gmail.com

intensive care units of the hospital. In the observational period, the hospital was exclusively designated for treating COVID-19 patients.

Of the total number of patients, 326 (7.4%) received transfusions. The patients who received transfusions were divided into two groups. The first group included 155 patients treated in the hospital intensive care units, while the second group included 147 subjects treated in the wards of the Bežanijska Kosa University Hospital Medical Center. The main criteria for ICU admission were radiographic or CT scan severity score progression, peripheral oxygen saturation (SpO_2) below 93% despite maximal conventional supportive oxygen therapy, laboratory test results, and arterial blood gas test. The study inclusion criteria were as follows: all patients with a verified diagnosis of COVID-19 (confirmed by a PCR test in real time), patients above the age of 18 years who met the criteria of disease severity for the third stage or higher (according to the National COVID-19 Guidelines) [13], and who required blood component transfusion. Criteria for the exclusion of patients from the study were incomplete data and patients transferred to other medical facilities.

In relation to the treatment outcome, data for 326 patients were stratified as deceased and survivors. Survivors were subjects dismissed from hospital. For all patients, data was collected from the electronic medical histories – the *Heliant* information system and the transfusion services operating protocol.

Data related to basic patient demographic characteristics (sex, age, comorbidities, therapy), laboratory parameters (hematological parameters: red blood cell count, white blood cell count, platelet count, hemoglobin levels, hematocrit, mean corpuscular volume, hemostatic parameters [prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer], biochemical parameters [C- reactive protein (CRP), urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, lactate dehydrogenase (LDH), ferritin], as well as data on oxygen support, were analyzed. All the data on administered transfusion therapy are related to the type and number of received blood component units; they were taken from the transfusion services protocol and relate to the application of concentrated erythrocytes and platelets, fresh frozen plasma (FFP), cryoprecipitate, and convalescent plasma.

Statistical analysis

The absolute and relative numbers, in percentages, were used for describing categorical data. Numerical data were described as the arithmetic mean with standard deviation or as the median with range, depending on data distribution. Normality of distribution was tested with mathematical and graphical methods. Two independent groups of subjects (patients with the COVID-19 infection treated in the intensive care units and patients with the COVID-19 infection treated in the hospital wards) were compared in relation to categorical and numerical variables. The difference in the distribution of categories of nominal data was determined by the χ^2 test. For testing the difference in the value of numerical data, Student's t-test or the Mann–Whitney U test were used, depending on the normality of distribution. All statistical methods were considered statistically significant at the significance level of 0.05. Analysis was performed using the IBM SPSS Statistics, Version 21.0 (IBM Corp., Armonk, NY, USA).

The study was approved by the Ethics Committee of the Bežanijska Kosa University Hospital Medical Center, Belgrade, Serbia (5425/1/).

RESULTS

The clinical characteristics of all patients who received transfusion therapy during hospitalization, the needs for transfusion therapy, and the treatment outcomes are presented in Table 1. Of the total number of all the transfused patients, 86% received erythrocytes, one-third of them received fresh frozen plasma, 10% received cryoprecipitate, while platelets were administered in around 6% of the patients. The mortality rate in the tested group was 46%.

Table 1. Characteristics of all patients who received a transfusion during their hospitalization due to COVID-19

Characteristic	Description
Age (years), $\bar{x} \pm$ sd	69.80 ± 13.85
Sex (male *vs.* female), n (%)	172 (52.8) *vs.* 154 (47.2)
Comorbidity (yes), n (%)	315 (96.6)
Cardiovascular diseases (yes), n (%)	254 (77.9)
Diabetes mellitus (yes), n (%)	108 (33.1)
Pulmonary diseases (yes), n (%)	19 (5.8)
Kidney disease (yes), n (%)	44 (13.5)
Gastrointestinal and/or liver diseases (yes), n (%)	47 (14.4)
Thyroid disease (yes), n (%)	20 (6.1)
Malignancies (yes), n (%)	73 (22.4)
Other comorbidities (yes), n (%)	27 (8.3)
Number of comorbidities, med (min–max)	2 (0–5)
Severity of clinical presentation (mild illness *vs.* severe illness), n %	175 (53.7) *vs.* 151 (46.3)
Oxygen support, n (%)	
Without	24 (7.4)
Intubation	148 (45.4)
Noninvasive ventilation	13 (4)
Oxygen therapy	141 (43.3)
Transfusion support, n (%)	326 (7.4)
Erythrocytes	279 (85.6)
Platelets	21 (6.4)
Fresh frozen plasma	95 (29.1)
Cryoprecipitate	33 (10.1)
Anti-COVID plasma	3 (0.9)
Length of hospital treatment (days), med (min–max)	18 (2–102)
Treatment outcome (discharged *vs.* lethal outcome), n (%)	175 (53.7) *vs.* 151 (46.3)

The clinical characteristics of the patients who received blood transfusion during their treatment in the intensive care units or the hospital wards are presented in Table 2.

Table 2. Comorbidities in COVID-19 patients who received a transfusion treated in the hospital intensive care units and wards

Parameter	Intensive care n = 175	Hospital wards n = 151	p*
Age (years), $\bar{x}$ ± sd	70.15 ± 15.44	69.50 ± 12.34	0.674
Sex (male *vs.* female), n (%)	105 (60) *vs.* 70 (40)	67 (44) *vs.* 84 (56)	**0.005**
Comorbidity (yes), n (%)	172 (98.3)	143 (94.7)	0.074
Cardiovascular diseases (yes), n (%)	144 (82.3)	110 (72.8)	**0.041**
Diabetes mellitus (yes), n (%)	60 (34.3)	48 (31.8)	0.633
Pulmonary diseases (yes), n (%)	9 (5.1)	10 (6.6)	0.570
Kidney disease (yes), n (%)	26 (14.9)	18 (11.9)	0.439
Gastrointestinal and/or liver diseases (yes), n (%)	22 (12.6)	25 (16.6)	0.307
Thyroid disease (yes), n (%)	11 (6.3)	9 (6)	0.903
Malignancies (yes), n (%)	31 (17.7)	42 (27.8)	**0.029**
Other comorbidities (yes), n (%)	14 (8)	13 (8.6)	0.842
Number of comorbidities, med (min–max)	2 (0–5)	2 (0–5)	0.712

*For the significance level of 0.05, according to the χ^2 test or the Mann–Whitney U test

Table 3. Hematological, hemostatic, and biochemical parameters prior to transfusion therapy

Parameters	Intensive care n = 175	Hospital wards n = 151	
Red blood cell count ($\times 10^{12}$/L) med (min–max)	2.6 (1.2–6.6)	2.8 (1.3–5.3)	**0.024**
White blood cell count ($\times 10^{9}$/L) med (min–max)	8.6 (0.05–175)	7.8 (0.2–86)	**0.013**
Platelet count ($\times 10^{9}$/L) med (min–max)	168.0 (1–631)	218.0 (13–843)	**0.002**
Hemoglobin (g/l) $\bar{x}$ ± sd	81.65 ± 22.88	82.70 ± 23.45	0.683
Hematocrit $\bar{x}$ ± sd	0.25 ± 0.07	0.26 ± 0.07	0.398
MCV (fL) $\bar{x}$ ± sd	88.89 ± 6.27	86.66 ± 10.32	**0.021**
INR med (min–max)	1.2 (0.8–10)	1.1 (0–11)	**< 0.001**
aPTT (s) med (min–max)	29.8 (17.6–558)	25.4 (0–230)	**< 0.001**
Fibrinogen (g/L) med (min–max)	3.7 (0.4 – 9.4)	3.7 (1.1–13.9)	**0.025**
D-dimer (ng/mL) med (min–max)	3,680 (203–35,200)	2,000 (23–26,720)	**< 0.001**
Direct bilirubin (μmol/L)	4.3 (1.2–169)	3.3 (1.2–395)	**0.001**
Total bilirubin (μmol/L)	11.9 (2.5–183)	8.7 (2.5–634)	**0.009**
AST (U/L)	43 (9–6500)	30.0 (7–481)	**< 0.001**
ALT (U/L)	30 (5–6484)	23 (5–309)	**0.001**
LDH (U/L)	792 (222–14,397)	520.0 (193–3943)	**< 0.001**
Ferritin (μg/L)	823 (36–4214)	461 (5–9125)	**< 0.001**
Urea (mmol/L)	10.6 (2.1–39)	7.9 (0–80.9)	**0.001**
Creatinine (μmol/L)	116 (29–600)	99 (43–647)	**0.043**
CRP (mg/L) med (min–max)	167.9 (11.9–571.4)	73.7 (1.8–408.1)	**< 0.001**

MCV – mean corpuscular volume; INR – international normalized ratio; aPTT – activated partial thromboplastin time; AST – aspartate aminotransferase; ALT – alanine aminotransferase; CRP – C-reactive protein; LDH – lactate dehydrogenase;
*for the significance level of 0.05, according to the Mann–Whitney U test

The distribution of the sexes was significantly different in the two examined groups (p = 0.005), with the male sex being more present amongst the patients in the intensive care units. The presence of cardiovascular diseases was significantly more common in COVID-19 patients with more severe clinical presentation treated in the intensive care units of the hospital (p = 0.041), while malignancies were more common in patients treated in the hospital wards (p = 0.029).

Table 3 presents the values of the hematological, hemostatic, and biochemical parameters prior to the application of transfusion therapy. Patients treated in the intensive care units had significantly lower red blood cell counts (p = 0.024) and platelet counts (p = 0.002), and significantly higher white blood cell counts (p = 0.013). Values of international normalized ratio (INR) (p < 0.001), aPTT (p < 0.001), and D-dimer (p < 0.001) were significantly higher in COVID-19 patients treated in the intensive care units, while the level of fibrinogen (p = 0.025) was higher in COVID-19 patients with a milder clinical presentation. All the tested biochemical parameters of liver function (direct bilirubin, p = 0.001; total bilirubin, p = 0.009; AST, p < 0.001; ALT, p = 0.001; LDH, p < 0.001; ferritin, p < 0.001), as well as the level of CRP (p < 0.001), were significantly elevated in COVID-19 patients treated in the intensive care units. Also, all evaluated parameters of kidney function (urea, p = 0.001; creatinine, p = 0.043) were significantly higher in the group of COVID-19 patients treated in the intensive care units, as compared to the patients treated in the hospital wards.

Table 4 presents the composition of the applied transfusion therapy. In the group of patients who received blood transfusion during their treatment in the intensive care units, 85% received erythrocytes, 39% received fresh frozen plasma, 19% received cryoprecipitate and 7% received platelets. A significantly higher number of red blood cell units was given to COVID-19 patients treated in the intensive care units. Also, a significantly greater number of COVID-19 patients treated in the intensive care units received FFP, as compared to patients treated in the hospital wards (39% *vs.* 18%, p < 0.001). The administration of cryoprecipitate was recorded only in patients treated in the intensive care units.

Treatment duration in relation to transfusion therapy is presented in Table 5. The treatment of patients who required red blood cell transfusion during their treatment for COVID-19 lasted significantly longer.

DISCUSSION

This study shows that 7.4% of all patients who were treated in hospital for COVID-19 required the administration of transfusion therapy. Our results are similar to the results published in previous studies, which have shown that transfusion application in COVID-19 patients is not very high, and ranges 6.2–13.4% [1, 8, 9]. The most commonly applied blood component were erythrocytes, which were indicated in 86% of the total number of all transfused patients. Our results are similar to the results from a previously published study by DeSimone et al. [12], where red blood cells made up for 83% of the total number of all administered blood components during

Table 4. Composition and doses of administered blood transfusions

Parameters	Intensive care n = 175	Hospital wards n = 151	p*
Number of patients who received erythrocytes, n (%)	148 (84.6)	131 (86.8)	0.576
Number of erythrocyte doses administered, med (min–max)	2 (1–21)	2 (1–11)	**0.005**
Number of patients who received platelets, n (%)	13 (7.4)	8 (5.3)	0.435
Number of platelet doses administered, med (min–max)	7 (4–13)	9 (5–32)	0.227
Number of patients who received fresh frozen plasma, n (%)	68 (38.9)	27 (17.9)	**< 0.001**
Number of fresh frozen plasma doses administered, med (min–max)	3 (1–23)	2 (0–10)	0.096
Number of patients who received cryoprecipitate, n (%)	33 (18.9)	0 (0)	**< 0.001**
Number of cryoprecipitate doses administered, med (min–max)	9 (5–47)	/	NA
Number of patients who received anti-COVID plasma, n (%)	2 (1.1)	1 (0.7)	0.650
Number of anti-COVID plasma doses administered, med (min-max)	2 (2–2)	2 (2–2)	1.000

*For the significance level of 0.05, according to the χ^2 test or the Mann–Whitney U test

Table 5. Treatment duration in relation to transfusion therapy in patients hospitalized due to COVID-19

Type of transfusion therapy	Treatment duration (days), med (min–max)		p*
	Yes	No	
Erythrocytes	18 (2–102)	15 (3–40)	**0.009**
Platelets	19 (7–42)	18 (2–102)	0.868
Fresh frozen plasma	18 (2–73)	17 (2–102)	0.656
Cryoprecipitate	20 (4–65)	17 (2–102)	0.264
Anti-COVID plasma	20 (19–30)	18 (2–102)	0.384

*For the significance level of 0.05, according to the Mann–Whitney U test

hospitalization. Our study showed that significantly greater number of red blood cell units was administered to patients treated in the intensive care units than to patients with a milder clinical presentation, who were treated in the hospital wards. A significantly greater number of patients in intensive care received FFP, as compared to patients treated in hospital wards (39% *vs.* 18%). Similar data can be found in studies by several authors, which have shown that the need for transfusion is significantly greater in patients treated in intensive care [1, 8, 10, 12].

Data analysis shows that the application of cryoprecipitate is indicated in 10%, while the application of concentrated platelets is indicated in 6% of all COVID-19 patients who received transfusion therapy during hospitalization. Anti-COVID plasma was given to three patients – two patients treated in the intensive care units and one patient treated in one of the hospital wards. The application of transfusions of platelets, FFP, and cryoprecipitate is low in COVID-19 patients who did not have major hemorrhage episodes, which was also confirmed in a study by Doyle et al. [14]. In our study, the percentage of transfused patients treated in the intensive care units was 18.2%, while the percentage of patients treated in the hospital wards who required transfusions was 4.9%. In a study by Grandone et al., the percentage of transfused patients in intensive care was 41.9%, while the percentage of transfused patients treated in the hospital wards was 10.3% [6], which is, in fact, significantly higher than in our study.

The mortality rate registered in our study, in the examined group, was 46%. These data are similar to the data from previous studies showing that mortality among COVID-19 patients who needed transfusions was between 34.8% and 45% [8]. According to the results of previously published studies, a great variation of mortality prevalence amongst COVID-19 patients admitted to hospital, which ranged 5.8–30.7%, was registered [15, 16, 17].

Almost all of our subjects had at least one comorbidity. Amongst these comorbidities, the most frequent ones were cardiovascular diseases and diabetes mellitus, which is in keeping with the data from previous studies [15, 18–22]. The presence of cardiovascular diseases was significantly more frequent in COVID-19 patients with a more severe clinical presentation who were treated in the intensive care units, while malignancies were more present in patients with a milder clinical presentation who were treated in the hospital wards. The male sex was more present in intensive care patients than in patients with a milder clinical presentation treated in the hospital wards. The presence of comorbidities did not affect the need for transfusion support in patients with the COVID-19 infection.

Earlier studies have shown that both sexes have the same susceptibility to the disease, but that men have the tendency to develop a more severe form of COVID-19 and are more likely to have a lethal outcome [23, 24, 25].

Our study showed that patients with a more severe clinical presentation treated in the hospital intensive care units had significantly lower red blood cell counts and platelet counts, and significantly higher white blood cell counts, as compared to COVID-19 patients with a milder clinical presentation treated in the hospital wards, which is in keeping with earlier studies [26, 27, 28]. The platelet count is significantly lower in patients with the more severe form of COVID-19. In COVID-19 patients, a low platelet count is connected with a higher risk of the severe form of disease and of mortality [26].

Controlling the parameters of hemostasis may help in determining the predictors of an unfavorable prognosis in patients with the COVID-19 infection. Tang et al. [29] reported that patients who had died of COVID-19 had significantly higher levels of D-dimer, longer PT, and longer aPTT, as compared to the surviving patients. The concentration levels of D-dimer in the serum, in patients with severe forms of COVID-19, were significantly higher than in patients with the milder form of the disease [26, 29].

Similarly to the data published in previous studies [26, 28], our study shows that patients with a more severe clinical presentation of COVID-19 had significantly higher levels of CRP, ferritin, LDH, AST, ALT, bilirubin, urea, and creatinine, as compared to patients with a milder form of the disease treated in the hospital wards.

The administration of FFP was in correlation with the values of INR, aPTT, bilirubin, urea, and ferritin. Patients who received cryoprecipitate had significantly higher values of INR, aPTT, D-dimer, AST, ALT, LDH, and ferritin, and significantly lower levels of fibrinogen, as compared to patients who did not receive cryoprecipitate. In a study by DeSimone et al. [12], high values of PT and aPTT were significantly linked with the need for transfusion, which was not the case with a low level of fibrinogen.

The average length of hospital treatment was 18 days, the shortest hospital stay was two days, while the longest was 102 days. In previous studies, the average length of hospital stay was between five and 29 days [30]. Our study showed that the treatment of patients who required red blood cell transfusion during their treatment for COVID-19 lasted significantly longer. However, it should be pointed out that these were patients with a more severe clinical presentation of the disease, whose recovery lasted longer.

As to the study limitations, it should be pointed out that this is a retrospective study and that certain parameters were not available for all the subjects. However, bearing in mind that the study comprehensively analyzes the administration of blood to COVID-19 patients, the results of this study may be a good indicator of the need for transfusion in this category of patients, which is important for planning and carrying out the collection and distribution of blood and blood components, especially during an epidemiological state of emergency.

CONCLUSION

Of the total number of COVID-19 patients hospitalized in the observed period, 7.4% received transfusion support therapy. The frequency of application of blood components was significantly higher in patients with the severe form of the disease. The presence of comorbidity did not affect the need for transfusion therapy. The registered mortality rate in the examined group was 46%; however, we cannot say with certainty whether and to what degree transfusion contributed to the registered mortality rate. To provide definite information about the cause–effect relationship, prospective studies are necessary.

Conflict of interest: None declared.

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Примена компонената крви код болесника лечених од ковида 19 – наша искуства

Војислава Милутиновић[1], Мирјана Ковач[2,3], Богдан Црнокрак[1,2], Марија Здравковић[1,2]

[1]Клиничко-болнички центар „Бежанијска коса", Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија;
[3]Институт за трансфузију крви Србије, Београд, Србија

САЖЕТАК

Увод/Циљ Постоји веома мало података о потреби за трансфузијом и њеном утицају на укупан морталитет болесника оболелих од ковида 19.

Циљ нашег истраживања је да се утврди потреба за трансфузијом крвних компонената код болесника лечених од ковида 19.

Методе Ретроспективна опсервациона студија обухватила је 4426 болесника позитивних на ковид 19 који су у периоду од 23. јуна 2020. до 2. маја 2021. године лечени у Клиничко-болничком центру „Бежанијска коса", од којих је 826 лечено у јединицама интензивне неге. Од укупног броја болесника, 326 (7,4%) болесника примало је трансфузију. Анализирани су клиничка слика, структура примењене трансфузиолошке терапије и лабораторијски параметри и исход лечења.

Резултати Од 828 болесника који су лечени у јединици интензивне неге, 151 (18,2%) болесник захтевао је трансфузију, док је проценат болесника лечених на одељењима који су захтевали трансфузију био 4,9%. Од укупног броја свих трансфундованих болесника, 86% је примило еритроците, трећина је примила замрзнуту свежу плазму, 10% је примило криопреципитат, док су тромбоцити примењени код 6% болесника. Утврђена стопа морталитета у испитиваној групи је 46%.

Закључак Учесталост примене крвних компонената је била значајно већа код болесника са тешким обликом болести. Присуство коморбидитета није утицало на потребу за трансфузиолошком терапијом. У групи болесника са тешком клиничком сликом лечених у јединицама интензивне неге, 85% болесника примило је еритроците, 39% примило је замрзнуту свежу плазму, 19% криопреципитат и 7% тромбоците.

Кључне речи: трансфузија; компоненте крви; ковид 19

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Effect of different demineralizing solutions and different exposing times on artificial initial caries lesion formation – an *in vitro* study

Milena Milanović[1], Zoran Mandinić[1], Jelena Juloski[1], Maja Dimitrijević[1], Biljana Miličić[2], Biljana Anđelski-Radičević[3], Vladimir Pavlović[4], Miloš Beloica[1]

[1]University of Belgrade, School of Dental Medicine, Clinic for Pediatric and Preventive Dentistry, Belgrade, Serbia;
[2]University of Belgrade, School of Dental Medicine, Department for Medical Statistics and Informatics, Belgrade, Serbia;
[3]University of Belgrade, School of Dental Medicine, Department for Biochemistry, Belgrade, Serbia;
[4]University of Belgrade, Faculty of Agriculture, Department for Physics and Mathematics, Belgrade, Serbia

Introduction/Objective Artificial enamel caries lesions are commonly created to simulate *in vivo* caries development and to examine the effect of non-invasive and microinvasive approaches in treatment of initial caries lesions.
The objective of the present study was to compare three different demineralizing solutions and exposing times in terms of the formation of artificial white spot lesions and to evaluate their demineralization effect through scanning electron microscopy observations.
Methods Twenty intact human premolars, extracted for orthodontic reasons, were thoroughly cleaned, stored in 0.1% thymol solution at room temperature and cut at the cementoenamel junction before demineralizing process. The specimens were randomly divided into three experimental groups, according to the used demineralization agent and the time of exposure: Group I (acetic acid; pH = 4.4; 96 hours); Group II (lactic acid; pH = 4.5; 120 hours); Group III (Lactic acid; pH = 4.3; 504 hours) and one control group (saline). After demineralisation, macroscopic appearance was checked and all specimens were observed under scanning electron microscope to evaluate the enamel characteristics and caries lesion depths.
Results In Group I and II enamel subsurface porosity with dissolution of enamel crystals is detected and the mean depths of white spot lesions were 48.55 μm (SD = 1.11) and 43.23 μm (SD = 6.74), respectively. In Group III structural integrity of enamel surface was not preserved.
Conclusion Demineralizing solutions used in experimental groups I and II resulted in artificial initial caries lesions with satisfactory characteristics and similar appearance on scanning electron microscopy. The outcome of demineralizing process which lasted 504 hours were cavitated enamel lesions.
Keywords: enamel; demineralization; white spot lesions; scanning electron microscopy

INTRODUCTION

Dental caries, one of the most common oral diseases worldwide, represents a major public health concern, as confirmed by the latest report on the Global Burden of Disease [1]. It is estimated that 2.5 billion people suffer from caries in permanent teeth, and more than 530 million children suffer from untreated caries in deciduous dentition [1, 2, 3]. Initial caries lesions, also known as white spot lesions (WSLs), are the earliest clinical signs of enamel caries. Subsurface enamel demineralization increases porosity and changes the optical characteristics of enamel. Consequently, the initial caries lesion appears as an opaque white spot, visibly different from the surrounding sound enamel due to differences in the refractive indexes (RI) [3, 4]. In the last decades, the prevalence of WSLs has increased, and some authors consider this increase a potential side effect of orthodontic therapy with fixed orthodontic appliances [5, 6]. If these lesions are not detected in the initial phase and are not properly managed in this reversible stage, they might progress into irreversible cavitated lesions. Additionally, WSLs might compromise the aesthetics of the anterior teeth [7, 8]. Contemporary dentistry established several non-invasive approaches in treatment of initial caries lesions to arrest caries lesions at an early stage and to improve its remineralization [9, 10, 11]. Fluoride-based and bioactive remineralizing agents such as casein phosphopeptide – amorphous calcium phosphate and calcium silicate-based materials are widely used [12–15]. As a modern, microinvasive approach, infiltration of the WSLs with a low viscosity composite resin, commercially available as ICON (DMG MORI, Hamburg, Germany), has been developed [16].

Accessible clinical experience points towards successful masking of whitish enamel discoloration in WSLs when treated with infiltrative resin [17]. However, there is still need for further examination of its impact on the other features and its effectiveness (such as surface roughness and enamel hardness, shear bond strength, penetration depth) [5]. *In vitro* studies

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Correspondence to:
Jelena JULOSKI
School of Dental Medicine
Clinic for Pediatric and Preventive Dentistry
dr Subotića Starijeg 11
11000 Belgrade
Serbia
jelena.juloski@stomf.bg.ac.rs

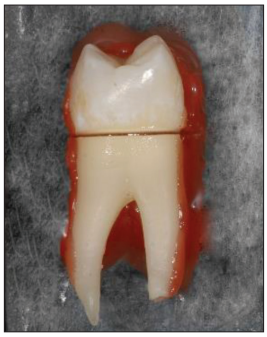

Figure 1. Before immersion into solutions, the roots of the teeth were separated at the cementoenamel junction with a low-speed diamond saw under water cooling; to perform the section, each tooth was fixed to glass plate using a sticky dental wax

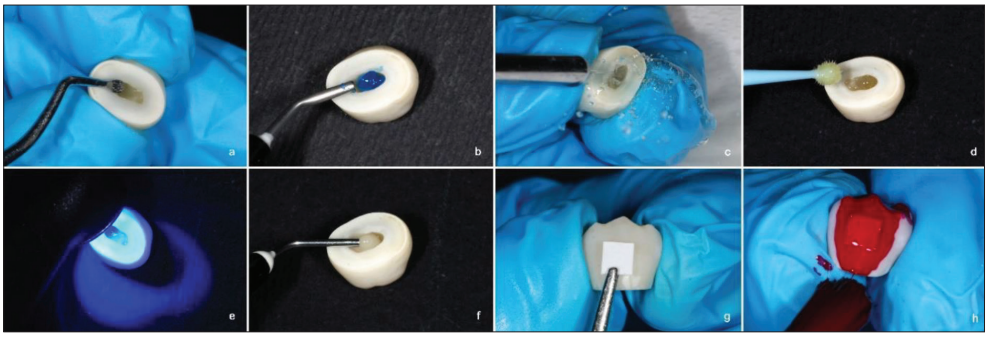

Figure 2. Preparation of the premolar crowns for the demineralizing process: a – pulp tissue was discharged; b – dentin was etched with 37% orthophosphoric acid; c – after 15 seconds, it was thoroughly rinsed and dried; d – an adhesive was applied on the dentin surface; e – polymerisation was performed; f – pulp chambers were obturated with flowable composite; g – the middle area of buccal and oral surface of all crowns was isolated using adhesive paper, sized 4 × 4 mm; h – the crowns were coated with an acid-resistant nail varnish

with resin infiltration of artificial initial caries lesions are a potential method to achieve this goal. There are numerous demineralization protocols for artificial WSLs which differ in the demineralization agents, the pH of the solutions/gels and the required time of the exposure to the demineralizing agent. They can contain lactic acid, acetic acid, methyl diphosphonate, acidified hydroxietilcellulose system, and in some of them fluoride might be added. The recent systematic review and meta-analysis reported that a duration of demineralization process, which simulated the formation of WSLs, varied from several to 1200 hours. In addition, pH of demineralizing solutions varied between four and five [5].

Developing adequate artificial initial caries lesion is needed to investigate the characteristics of different remineralizing protocols. Therefore, the objective of the present study was to compare the effect of three different demineralizing solutions and exposure times on the formation of artificial WSLs and evaluate their demineralizing effects through scanning electron microscopy (SEM).

METHODS

The present study was conducted in the Clinic for Pediatric and Preventive Dentistry and Department for Biochemistry, School of Dental Medicine, University of Belgrade and SEM evaluation was performed in the Department for Physics and Mathematics, Faculty of Agriculture, University of Belgrade.

The present work was approved by competent ethics committee (Ethics Committee of School of Dental Medicine, University of Belgrade, no 36/41) and conforms to the legal standards.

Twenty caries-free permanent maxillary or mandibular, first or second human premolars extracted upon orthodontic indication were used for this study. The teeth were visually examined using LED light to ensure that there are no enamel surface defects or microcracks.

Specimen preparation

The selected teeth were cleaned with periodontal curettes to remove any remaining soft tissue and with fluoride free prophylactic paste (Cleanic, Kerr, Orange, CA, USA), applied by a brush mounted in a low-speed hand piece, under water cooling. Subsequently, the teeth were washed in distilled water and stored in 0.1% thymol solution at room temperature until further use for no longer than 1 month.

Before demineralization process, roots were removed at the cementoenamel junction with a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling (Figure 1). Pulp tissue was removed, and pulp chambers of the premolar crowns were obturated with flowable composite (Flow-It ALC, Pentron). The premolar crowns were randomly divided into three experimental groups and one control group (each group containing five teeth).

All crowns in experimental groups were coated with an acid-resistant nail varnish (Essence, Cosnova GmbH, Zulcbah, Germany), except for a middle area of buccal and oral surfaces which were isolated using adhesive paper, sized 4 × 4 mm (Figure 2). The adhesive papers were removed after the nail varnish had dried completely at room temperature. Each crown of the tooth gave two specimens (buccal and oral half), stored in saline solution at room temperature until SEM evaluation (Figure 3).

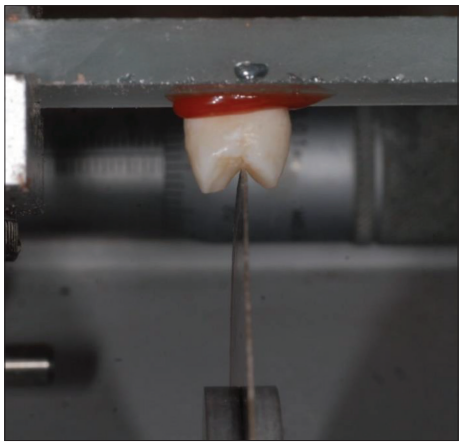

Figure 3. After demineralization, all premolar crowns were sectioned into two halves (buccal and oral) with a low-speed diamond saw under water cooling

Artificial initial caries lesions

The specimens were randomly divided into three experimental groups, according to the used demineralization agent, and one control group (n = 10):

Group I – demineralizing solution I – acetic acid based; pH = 4.4; for 96 hours [10, 18, 19];

Group II – demineralizing solution II – lactic acid based; pH = 4.5; for 120 hours [20];

Group III – demineralizing solution III – lactic acid based; pH = 4.3; 504 hours [21];

Group IV – control group (stored in saline solution at room temperature until SEM evaluation).

The composition of each demineralizing solution, its pH and exposure times are reported in Table 1.

Table 1. Characteristics of solutions used for demineralization

Features	Group I	Group II	Group III
Composition	2.2 mmol/L calcium chloride	12 mmol/L calcium chloride	18 mmol/L calcium chloride
	2.2 mmol/L monopotassium phosphate	10 mmol/L monopotassium phosphate	7.8 mmol/L monopotassium phosphate
	0.05 mmol/L acetic acid	50 mmol/L lactic acid	100 mmol/L lactic acid
	/	100 mmol/L sodium chloride	3 mmol/L sodium azide
pH	4.4	4.5	4.3
Exposure time	4 days / 96 hours	5 days / 120 hours	21 days / 504 hours

Each specimen was immersed in 20 ml of appropriate demineralizing solution in a sterile plastic container. The pH of demineralizing solutions was checked every day with a probe to maintain the initially defined values (Mi 150, pH/Temperature Bench Meter, Martini instruments; Figure 4). If needed, it was adjusted with potassium hydroxide. In Group I and Group III, specimens were stored at room temperature [10, 18, 19] and in Group II they were stored in 37°C incubator (Binder Gmbh Incubator, Tuttlingen, Germany) [21].

Subsequent to the artificial demineralization, nail varnish was carefully removed with oil-free acetone from the specimens, and they were thoroughly rinsed with distilled water. All specimens were visually inspected and macroscopic appearance of enamel lesion was evaluated.

Scanning electron microscopy evaluation of artificial white spot lesions

After completed demineralization procedure, all premolar crowns were sectioned into two halves (buccal and oral) with a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling (Figure 3). In order to perform the section, each crown was fixed to glass plate using sticky dental wax (Galeo, Galenika, Belgrade, Serbia).

From obtained buccal and oral premolar halves, sections were made through the artificially created WSLs in experimental groups and from sound enamel in the control group. Each premolar half was sectioned in vestibulo-oral direction, into 1-mm-thick slices with a low-speed diamond saw (Isomet, Buehler Ltd, Lake Bluff, IL, USA) under water cooling. Sections were made through the middle area and two slices were obtained from each premolar half. All slices were polished with silicon carbide paper (600, 800, 1000, 1200 grit paper) under water cooling, rinsed with distilled water, dried and etched with phosphoric acid gel (Gel Etchant, Kerr) for 30 seconds. After abundant rinsing and drying, the specimens were dehydrated in an ascending ethanol series (25%, 50%, 70%, 90%, 95%). Drying was performed with oil free air spray. Specimens were mounted on metallic stubs, gold-sputtered (Bal-Tec SCD 005 Sputter Coater) and observed under a Scanning Electron Microscope (JEOL-JSM-6460LV, Tokyo, Japan) under different magnifications (40, 100, 700, 1000, 1500, 4000) to evaluate the characteristics of initial caries lesions – demineralization depth and pattern.

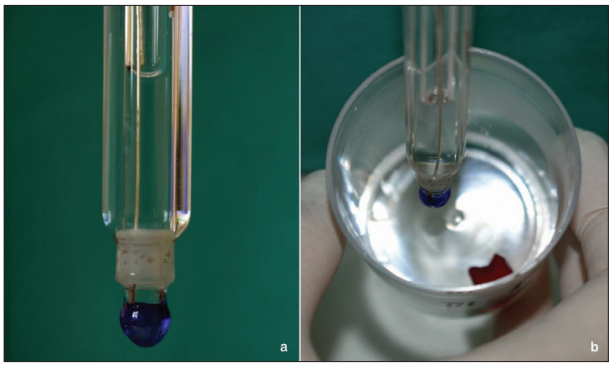

Figure 4. The pH of the demineralizing solutions was evaluated daily to maintain the initially defined values

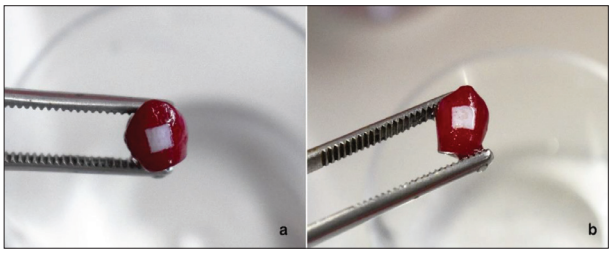

Figure 5. The areas exposed to the demineralizing solution appeared whitish-opaque on daily light at the end of the process

RESULTS

In the first and the second experimental group, areas exposed to the demineralizing solution appeared whitish-opaque on daily light at the end of demineralization process. In both groups, visual inspection of the specimens did not reveal any localized breakdown of the enamel (Figure 5). On the other hand, in the third experimental group the structural integrity of the enamel was not maintained.

Sound enamel in the control group (Group IV) on cross-sectional SEM micrograph showed physiological enamel rod appearance (Figure 6). The surface of sound enamel might appear with occasional depressions,

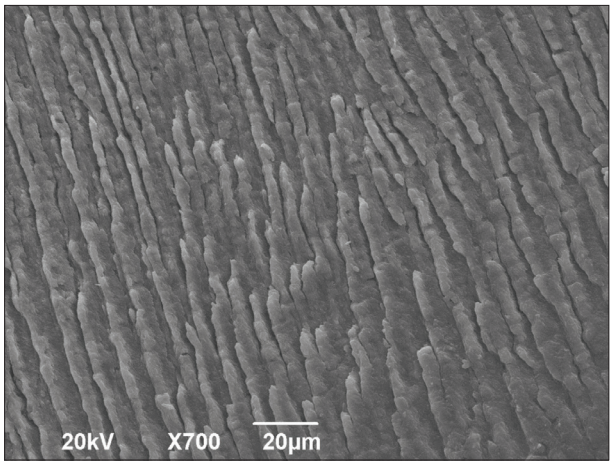

Figure 6. Scanning electron microscopy evaluation showed physiological enamel rod appearance in the control group

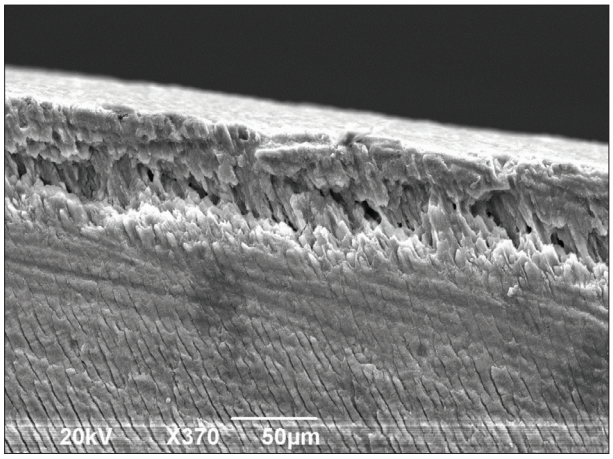

Figure 7. In the experimental group I enamel subsurface porosity with dissolution of enamel crystals was detected

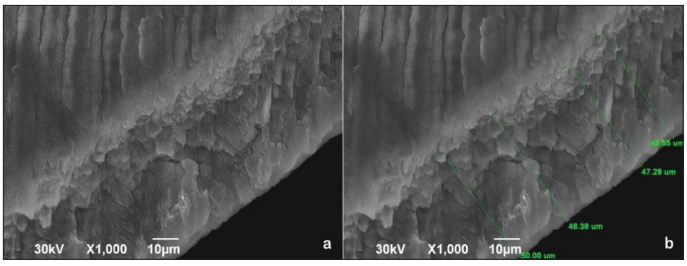

Figure 8. The depth of formed initial caries lesions was evaluated through scanning electron microscopy observations

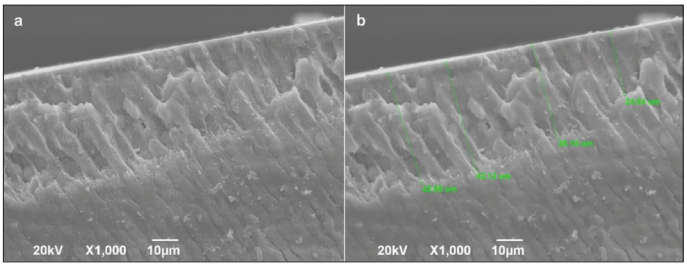

Figure 9. In the experimental group II caries-like subsurface lesions were obtained; they had similar enamel appearance as in the first experimental group

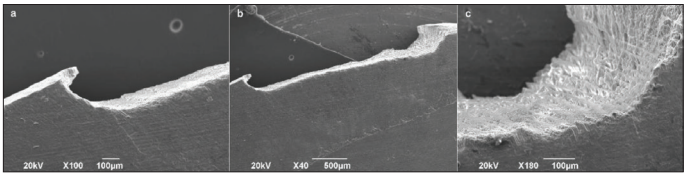

Figure 10. In the experimental group III structural integrity of enamel surface was not preserved; surface erosion with prismatic pattern of enamel surface dissolution can be seen

representing prism ends termination at the surface. These structures were usually located at the base of perikymata. The present SEM evaluation showed relatively smooth enamel without these morphological variations.

In Group I enamel subsurface porosity with dissolution of enamel crystals was detected (Figure 7). It indicated formation of caries-like subsurface lesion. The mean depth of lesion was 48.55 µm (SD = 1.11) (Figure 8). In Group II, the mean depth of the lesion was 43.23 µm (SD = 6.74). In Figure 9 similar enamel appearance as in the previous group was found. The appearance of these caries-like lesions might be similar to the surface of sound enamel except for surface depressions. Furthermore, in both groups (Group I and Group II), small number of specimens on microphotographs showed that some surface enamel areas of the WSLs were eroded while surrounded enamel surface was intact.

In the Group III structural integrity of enamel surface was not preserved. Figure 10 presents surface erosion with prismatic pattern of enamel surface dissolution. Also, this lactic acid based demineralizing solution resulted in loss of enamel prism cores with present porosities and preserved enamel prism peripheries.

DISCUSSION

Artificial enamel caries lesions are commonly created to simulate *in vivo* caries development. Enamel specimens are usually exposed to demineralizing solutions and gels composed of acetic or lactic acid, undersaturated regarding hydroxyapatite, to simulate the plaque fluid conditions and allow the formation of initial enamel lesions [22, 23].

Freitas et al. [24] used methylcellulose gel. Its high viscosity could compromise the diffusion rate of the acid, resulting in a reduced demineralization and fewer pores.

Issa et al. [25] concluded that presence of fluoride in the demineralizing process, especially when gel was used, significantly reduced the lesion depth and mineral loss in permanent teeth. In addition, typical subsurface lesions could be formed in the absence of fluoride. So, in the present study, demineralizing solutions without fluoride were used.

Considering previously mentioned solutions and availability of solutions' components, which is an important contributing factor in selection of demineralization agents [26], acetic and lactic acid-based protocols were selected for the present study. pH values of the solutions used in the study are also in accordance with a recently published systematic review and meta-analysis suggesting that the pH of demineralizing solutions should vary between four and five [5].

When it comes to duration of demineralizing process, less consistent findings are present in literature. As previously mentioned, this period varies from few to 1.200 hours. In the present study, solution used in the first experimental group lasted 96 hours as reported in several *in vitro* experiments [10, 18, 27]. Also, Behrouzi et al. [19] used solution for the same time, but it was acidified hydroxietilcellulose system. Group II in the present study had longer exposure time (120 hours).

On the other hand, in the third experimental group, it was significantly longer (21 day / 504 hours), as this protocol was suggested by Prajapati et al. [21]. These authors reported the mean depth of formed lesions of 341 μm. In the present pilot study, initial caries lesion with subsurface demineralization were not formed by this demineralizing solution. On the contrary, it resulted in localized enamel breakdowns with areas of prismatic pattern of dissolution.

The mean depth of artificial initial caries lesions obtained in the present study was 48.55 μm in the first group and 43.23 μm in the second group, which is not as deep as reported in some *in vitro* studies [10, 21], although there are similar findings in literature. Magalhães et al. [22] reported mean depth of subsurface enamel lesions between 35 and 52 μm, except for methyl diphosphonate lesions (86 μm).

In the present experiment, WSLs were visually detectable on the wet specimens and some authors stated that WSL was visible on a wet tooth surface when it had penetrated deeper into the enamel surface [28]. They could be described as whitish areas with rough enamel surface enamel.

Artificial WSLs induced in this experiment (Group I and Group II) demonstrated intact surface layer with subsurface prismatic demineralization and an enlargement of the prism sheaths. In some specimens, two types of enamel surface involvement were described: areas of erosion and other areas apparently intact, when compared to the sound enamel surrounding them. These two distinct sites of the WSL also presented diverse levels of enamel dissolution. Eroded areas showed irregular patterns of surface destruction, and, by some authors, they indicated an advanced stage of the dissolution of the enamel promoted by the carious process [29].

CONCLUSION

Based on the results of this study it could be concluded that different demineralizing solutions produce artificial initial caries lesions of variable features and depths. Demineralizing solution based on acetic acid of pH value 4.4- and 96-hour-long exposure time and solution based on lactic acid of pH value 4.5 and 120 hours exposure time provide artificial WSLs with satisfactory characteristics. The outcome of demineralizing process which lasted 21 days were cavitated enamel lesions.

Conflict of interest: None declared.

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Утицај различитих раствора за деминерализацију и времена излагања на настанак и карактеристике почетних каријесних лезија глеђи – *in vitro* студија

Милена Миланović[1], Зоран Мандинић[1], Јелена Јулоски[1], Маја Димитријевић[1], Биљана Миличић[2], Биљана Анђелски-Радичевић[3], Владимир Павловић[4], Милош Белоица[1]

[1]Универзитет у Београду, Стоматолошки факултет, Клиника за дечју и превентивну стоматологију, Београд, Србија;
[2]Универзитет у Београду, Стоматолошки факултет, ОЈ институтски предмети, наставни предмет Информатика у стоматологији, Биостатистика, Београд, Србија;
[3]Универзитет у Београду, Стоматолошки факултет, ОЈ институтски предмети, наставни предмет Биохемија, Београд, Србија;
[4]Универзитет у Београду, Пољопривредни факултет, Одељак за физику и математику, Београд, Србија

САЖЕТАК

Увод/Циљ Почетна каријесна лезија глеђи формирана у *in vitro* условима се често користи за испитивање ефикасности различитих средстава за реминерализацију.

Циљ овог истраживања био је испитивање утицаја три различита раствора за деминерализацију и различитог времена излагања на формирање белих мрља, као и процена карактеристика насталих почетних каријесних лезија глеђи помоћу скенирајућег електронског микроскопа.

Методе У овом *in vitro* истраживању коришћено је 20 премолара хуманог порекла који су извађени из ортодонтских разлога. Након детаљног уклањања остатака ткива, потопљени су у 0,1% раствор тимола, на собној температури. Пре процеса деминерализације извршено је одсецање корена у нивоу глеђноцементне границе. Методом случајног избора разврстани су у три експерименталне групе, у зависности од употребљеног раствора за деминерализацију и времена у току којег су били изложени дејству раствора: Група 1 (сирћетна киселина; *pH* = 4,4; током 96 сати); Група 2 (млечна киселина; *pH* = 4,5; 120 сати); Група 3 (млечна киселина; *pH* = 4,3; 504 сата) и контролна група (физиолошки раствор). Пресеци здраве глеђи у контролној групи и особине насталих каријесних лезија глеђи у експерименталним групама посматрани су на скенирајућем електронском микроскопу.

Резултати У првој и другој експерименталној групи уочен је настанак почетних каријесних лезија глеђи, са потповршинским зонама деминерализације и повећане порозности. У трећој експерименталној групи није очуван интегритет површине глеђи и дошло је до настанка кавитета.

Закључак Раствори за деминерализацију употребљени у првој и другој експерименталној групи дали су почетне каријесне лезије сличних особина, док је исход трећег раствора био настанак дефеката глеђи.

Кључне речи: глеђ; деминерализација; почетне каријесне лезије глеђи; скенирајући електронски микроскоп

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Open retropubic radical prostatectomy versus external beam radiation therapy for localized prostate cancer – patient-reported outcomes

Dragoslav Bašić[1,2], Aleksandar Skakić[1,2], Miloš Stević[1,3], Aleksandra Ignjatović[1,4], Žarko Mirković[5], Ivan Ignjatović[1,2], Jovan Janić[2], Andrej Veljković[6], Ljubinka Janković-Veličković[1,7], Jovan Hadži-Đokić[8]

[1]University of Niš, Faculty of Medicine Niš, Niš, Serbia;
[2]University Clinical Center of Niš, Clinic for Urology, Niš, Serbia;
[3]University Clinical Center of Niš, Center for Nuclear Medicine, Niš, Serbia;
[4]University of Niš, Faculty of Medicine, Department of Medical Statistics and Informatics, Niš, Serbia;
[5]University Clinical Center of Niš, Clinic for Oncology, Niš, Serbia;
[6]University of Niš, Faculty of Medicine, Department of Biochemistry, Niš, Serbia;
[7]University Clinical Center of Niš, Pathology and Pathological Anatomy Center, Niš, Serbia;
[8]Serbian Academy of Sciences and Arts, Belgrade, Serbia

SUMMARY

Introduction/Objective Active treatment options for localized prostate cancer (LPCa) include surgery and radiotherapy with androgen deprivation therapy (ADT) in selected cases, but all options have side effects, mainly addressed to urinary, sexual, and bowel function.

Our study aimed to assess and compare patient-reported outcome measures (PROMs) after open retropubic radical prostatectomy (ORRP) or external beam radiotherapy (EBRT).

Methods Between June 2019 and May 2021, a total of 120 patients, with LPCa had undergone active treatment, as follow: ORRP – 60 patients and EBRT – 60 patients. A validated questionnaire, the Expanded Prostate Cancer Index Composite Short Form (EPIC-26) instrument was used to assess PROM, through the following domains: urinary, sexual and bowel. Patients completed a questionnaire at baseline and six, 12, and 24 months after primary treatment.

Results All urinary scores had statistically significant interaction between time and group. After six, 12, and 24 months, all urinary scores were statistically significantly lower in the ORRP group. After 12 and 24 months, bowel score values were statistically significantly lower in patients in the ERBT group. Sexual scores change statistically significant during the follow-up period, without difference between the groups ($p < 0.05$).

Conclusion Both ORRP and EBRT are associated with decline of sexual scores. ORRP showed significant variations in all urinary scores, with more pronounced negative impact on urinary symptoms compared to EBRT during the entire follow-up period. Bowel scores are lower in EBRT.

Keywords: localized prostate cancer; open retropubic radical prostatectomy; external beam radiotherapy; patient-reported outcomes

INTRODUCTION

Prostate cancer represents the most common noncutaneous malignancy in men [1]. Its annual share accounts for 7.1 % of all cancers detected, with rising trend nowadays [2, 3]. According to the latest epidemiological data for the male population, in 2023, the most common malignancies were prostate, lung and colorectal cancers, which accounted for 48% of all cases, while prostate cancer alone had shared with 29% [4]. At the time of prostate cancer diagnosis, 77% of patients have localized disease [5]. However, it was observed that since 2014, a 3% annual increase in the incidence of prostate cancer has been associated with a 4.5% annual increase in cases of higher grade, with locally advanced or high-stage disease [6].

Nevertheless, prostate cancer screening and other improvements in the diagnostic and therapeutic procedure has led to sustained declining trend in annual prostate cancer mortality rates, from 4% in 1994 to 0,6% nowadays [7]. Recent data demonstrated that five-year relative survival rate of prostate cancer is 97%, and is one of the highest among all malignancies [8]. Since the prostate cancer has a long natural history and is age-related, it has become evident that non-cancer comorbidities in patients with prostate cancer represent important danger, causing 57% of all deaths [9, 10].

Active treatment options for localized prostate cancer (LPCa) include surgery (radical prostatectomy) and radiotherapy [external beam radiotherapy (EBRT), or brachytherapy] with androgen deprivation therapy (ADT) in selected cases, but all options have side effects, mainly addressed to urinary, sexual, and bowel function [11]. Despite the fact that cancer-free survival is an essential measure of therapeutic success, the patient's perception of health-related quality of life (HRQoL) represents important issue [12]. Various patient-reported outcome measures (PROMs) are used to assess side

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Correspondence to:
Dragoslav BAŠIĆ
University of Niš
Faculty of Medicine
University Clinical Center of Niš
Clinic for Urology
Niš 18000
Serbia
basicdr@gmail.com

effects and symptoms, and to evaluate HRQoL [9]. Our study aimed to assess and compare HRQoL in patients who underwent open retropubic radical prostatectomy (ORRP) or external beam radiotherapy (EBRT), using Expanded Prostate Cancer Index Composite (EPIC) PROM.

METHODS

Between June 2019 and May 2021, a total of 120 patients, with LPCa had undergone active treatment through the following procedures:

1. Group ORRP – 60 patients, mean age 64 (48–73) years, who underwent ORRP;
2. Group EBRT – 60 patients, mean age 71 (63–80) years, who underwent EBRT.

All 120 patients were diagnosed with clinically LPCa, through the following procedures: prostate-specific antigen (PSA) testing, digital rectal examination of the prostate, transrectal ultrasound-guided biopsy of the prostate, histopathological examination of specimens, multislice computerized abdomino-pelvic tomography and bone scintigraphy.

Indications for ORRP were: PSA ≤ 20 ng/ml, or Gleason score ≤ 7 (ISUP grade ≤ 2/3), or clinical stage ≤ T2b (for low- and intermediate-risk PCa); PSA > 20 ng/ml, or Gleason score > 7 (ISUP grade ≤ 4/5), or clinical stage ≤ T2c (for high-risk PCa), ECOG performance status 0 or 1, aged ≤ 70 years (except in selected cases with life expectancy of > 10 years) [11]. Contraindications were as follows: life expectancy ≤ 10 years, medical history of malignancies, end-stage renal disease, kidney transplantation and advanced cardiovascular or respiratory diseases. Indications for EBRT included high-risk PCa, Gleason score > 8 or PSA > 20 ng/mL, patient's motivation, contraindications for ORRP, and advanced age.

After the histopathological confirmation of prostate cancer, all patients were examined at the Council of Urological Oncology, when the appropriate therapeutic procedure was proposed. Upon acceptance of the proposal, the patients received the Council's decision and an informed consent form. Treatment began 6–8 weeks after the Council's decision. We used the Walsh operative technique in all patients in the ORRP group [13]. EBRT was delivered at a dose of 74 Gy, in 37 fractions over six weeks, with three-dimensional conformal radiation therapy (3D-CRT).

A validated questionnaire, the Expanded Prostate Cancer Index Composite Short Form (EPIC-26) instrument was used to assess PROM, through the following domains: urinary, sexual, and bowel [14]. Patients completed a questionnaire regularly before prostate biopsy and six, 12 and 24 months after primary treatment.

Statistical data processing was performed in the R software package (R Core Team, R Foundation for Statistical Computing, Vienna, Austria). Data are presented as arithmetic mean and standard deviation. The comparison of the values of the tested scores in the monitoring period in relation to the groups was performed by ANOVA for repeated measures. If a statistically significant time x group interaction was obtained, the t-test or Mann–Whitney test was used to compare simple effects. The null hypothesis was tested with a significance threshold of $p < 0.05$.

This work is conducted according to the Declaration of Helsinki ethical principles, with guaranteed discretion of personal data, and was approved by the Ethics Committee of the Faculty of Medicine of the University of Niš (No. 12-8818-2/8).

RESULTS

According to the results of the t-test (Table 1) it is noticed that there is a statistically significant difference in the age between observed groups of patients (*t*-statistics = 2.421; p-value = 0.017), in favor of EBRT group. Table 1 shows mean age of patients in study groups.

Table 2 shows the values of urinary scores in relation to the examined groups during the follow-up period. It was found that for all investigated urinary scores there is a statistically significant time x group interaction ($p < 0.05$). Before treatment, all urinary scores differed between the groups, except for incontinence and urinary irritative/obstructive score (UIO). After six, 12, and 24 months, all urinary scores were statistically significantly lower in the ORRP group compared to ERBT ($p < 0.05$). Values of urinary score in relation to the studied groups during the 24 month-follow-up are shown in Figure 1.

Table 3 shows the values of bowel scores in relation to the examined groups during the follow-up period. It was found that there is a statistically significant time × group interaction for all examined bowel scores ($p < 0.05$). Before treatment, bowel score values did not differ between groups ($p = 0.422$, $p = 0.304$, $p = 0.528$). Even after six months, the values of bowel scores do not differ between the groups ($p = 0.228$, $p = 0.136$, $p = 0.329$). After 12 months, bowel score values were statistically significantly lower in patients in the ERBT group compared to the ORRP group ($p = 0.014$, $p = 0.006$ and $p = 0.029$). After 24 months, bowel score values were statistically significantly lower in patients in the ERBT group compared to the ORRP group ($p = 0.011$, $p = 0.003$ and $p = 0.029$). Values of bowel score in relation to the studied groups during the 24 month-follow-up are shown in Figure 2.

The total sexual score, sexual function and sexual bother change statistically significant during the follow-up period ($p < 0.001$ for all) (Table 4). There is no statistically significant difference between the groups ($p = 0.800$, $p = 0.634$, $p = 0.856$) and there is no significant interaction time x group ($p = 0.164$, $p = 0.312$, $p = 0.104$). The movement of the total scores in relation to the examined groups in a period of 24 months is shown in Figure 3.

DISCUSSION

In the present study, we evaluated patients' PROMs using the EPIC-26 instrument, which has been most frequently applied in clinical practice [9].

Table 1. Independent samples t-test for equality of means

Variable	Mean of group ORRP (N = 60)	Mean of group EBRT (N = 60)	Difference	Std. Error Difference	t	df	p-value	95% confidence interval	
								Lower	Upper
Age	64 (48–73)	71 (63–80)	7.000	2.891	2.421	118	0.017	1.2749	12.7251

ORRP – open retropubic radical prostatectomy; EBRT – external beam radiation therapy

Table 2. Urinary score values in relation to the examined groups in the follow-up period

Score	Group	Before treatment	Six months	12 months	24 months	p
Urinary summary	ORRP group	78.27 ± 6.82	69.57 ± 13.09	72.53 ± 11.15	76.07 ± 12.05	< 0.001[1] < 0.001[2] < 0.001[3]
	ERBT group	82.76 ± 6.16	81.79 ± 7.83	81.41 ± 8.74	85.54 ± 7.67	
Urinary function	ORRP group	98.04 ± 4.29	78.4 ± 20.83	79.4 ± 19.6	79.06 ± 19.78	< 0.001[1] < 0.001[2] < 0.001[3]
	ERBT group	95.71 ± 6.54	93.37 ± 11.09	93.71 ± 11.13	93.71 ± 11.13	
Urinary bother	ORRP group	64.15 ± 10.42	63.26 ± 9.98	67.62 ± 8.54	73.93 ± 8.51	< 0.001[1] 0.002[2] < 0.001[3]
	ERBT group	73.51 ± 7.87	73.51 ± 7.87	72.62 ± 9.61	79.7 ± 7.88	
Incontinence	ORRP group	96.4 ± 9.02	62.27 ± 34.92	65.29 ± 32.29	65.91 ± 32.24	< 0.001[1] < 0.001[2] < 0.001[3]
	ERBT group	95.26 ± 10.31	92.34 ± 16.22	90.99 ± 18.46	92.03 ± 17.8	
Urinary irritative / obstructive	ORRP group	75.05 ± 7.72	79.63 ± 5.92	83.1 ± 7.61	87.74 ± 5.23	< 0.001[1] < 0.001[2] 0.008[3]
	ERBT group	77.14 ± 5.63	77.14 ± 5.63	77.56 ± 8.37	84.52 ± 6.92	

Repeated measures ANOVA, [1]time effect, [2]interaction time × group, [3]group effect;
ORRP – open retropubic radical prostatectomy; EBRT – external beam radiation therapy

Table 3. Bowel score values in relation to the examined groups in the follow-up period

Score	Group	Before treatment	Six months	12 months	24 months	p
Bowel summary	ORRP group	95.18 ± 14.59	95.18 ± 14.59	95.18 ± 14.59	95.18 ± 14.59	0.003[1] 0.003[2] 0.052[3]
	ERBT group	92.83 ± 17.24	91.28 ± 20.17	85.83 ± 25.07	85.48 ± 24.94	
Bowel function	ORRP group	95.95 ± 12.66	95.95 ± 12.66	95.95 ± 12.66	95.95 ± 12.66	0.002[1] 0.002[2] 0.020[3]
	ERBT group	93.39 ± 14.42	91.73 ± 17.77	86.61 ± 22.3	85.89 ± 22.06	
Bowel bother	ORRP group	94.4 ± 16.61	94.4 ± 16.61	94.4 ± 16.61	94.4 ± 16.61	0.005[1] 0.005[2] 0.101[3]
	ERBT group	92.26 ± 20.29	90.83 ± 22.85	85.06 ± 28.13	85.06 ± 28.13	

Repeated measures ANOVA, [1]time effect, [2]interaction time × group, [3]group effect;
ORRP – open retropubic radical prostatectomy; EBRT – external beam radiation therapy

Table 4. Sexual score values in relation to the examined groups in the follow-up period

Score	Group	Before treatment	Six months	12 months	24 months	p
Sex summary	ORRP group	58.94 ± 28.76	47.63 ± 26.79	39.51 ± 19.69	42.76 ± 21.47	< 0.001[1] 0.164[2] 0.800[3]
	ERBT group	53.64 ± 28.02	47.26 ± 25.19	41.41 ± 22.59	42.45 ± 22.04	
Sex function	ORRP group	57.63 ± 29.32	45.98 ± 27.45	35.91 ± 19.74	40.42 ± 22.89	< 0.001[1] 0.312[2] 0.634[3]
	ERBT group	51.92 ± 29.5	44.42 ± 26.59	37.73 ± 22.66	38.4 ± 22.89	
Sex bother	ORRP group	61.88 ± 28.83	51.35 ± 27.16	47.60 ± 25.08	48.02 ± 25.12	< 0.001[1] 0.104[2] 0.856[3]
	ERBT group	57.5 ± 27.49	53.65 ± 29.46	49.69 ± 30.06	51.56 ± 29.15	

Repeated measures ANOVA, [1]time effect, [2]interaction time × group, [3]group effect,
ORRP – open retropubic radical prostatectomy; EBRT – external beam radiation therapy

Barocas et al. [15] analyzed PROMs based on the EPIC instrument, after observation, EBRT or radical prostatectomy (RP) in 2750 patients with localized PCa. The effects of RP were associated with lower urinary incontinence (UI) and sexual function scores compared to EBRT, except for the bowel score which was better at 12 months. In a recently published study on PROMs after surgery or irradiation in LPCa, Hashin et al. [16] reported significantly lower urinary scores in surgically treated patients and significantly lower bowel scores in irradiated patients, while in the follow-up period there was a decrease in the difference in both domains. In the sexual domain, a decrease in the score after surgical treatment was reported, while the score was unchanged after irradiation. Analyzing PROMs in 1141 patients after RP, EBRT, permanent prostate brachytherapy (PPB) and Active Surveillance (AS), Chen et al. [17] concluded that the UI score was the lowest after RP, urinary bother and bowel scores after EBRT, while after three months the sexual score was worse after RP compared to EBRT. After 24 months, there were no statistically significant differences in relation to the analyzed domains.

However, the curative potential of RP and EBRT is to some extent compromised by post-interventional complications and consequent symptoms, with urinary, sexual,

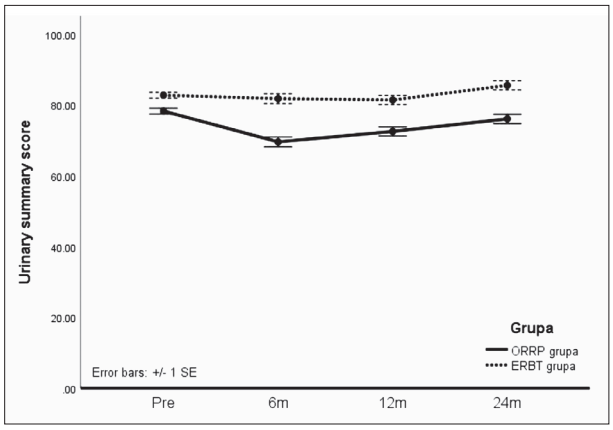

Figure 1. Values of urinary score in relation to the studied groups during the 24 month-follow-up; ORRP – open retropubic radical prostatectomy; EBRT – external beam radiation therapy

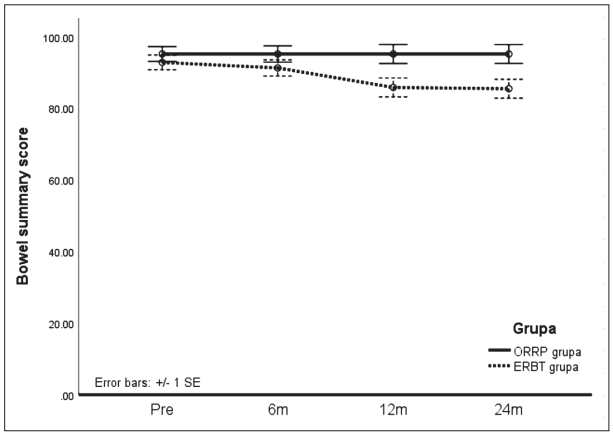

Figure 2. Values of bowel score in relation to the studied groups during the 24 month-follow-up; ORRP – open retropubic radical prostatectomy; EBRT – external beam radiation therapy

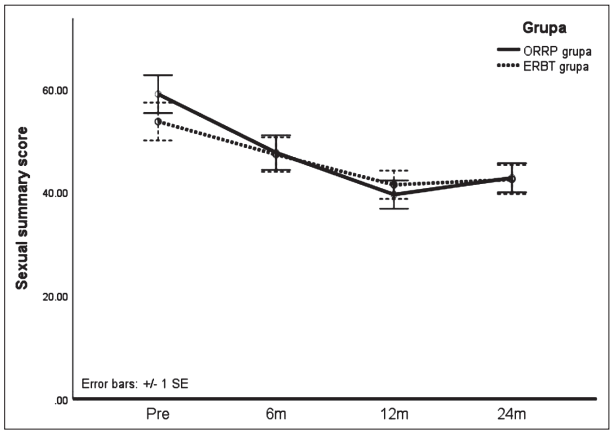

Figure 3. Values of sexual score in relation to the studied groups during the 24 month-follow-up; ORRP – open retropubic radical prostatectomy; EBRT – external beam radiation therapy

and intestinal most pronounced. Symptoms of erectile dysfunction (ED) and UI have been addressed to surgery, while bowel and irritative urinary symptoms are predominantly associated with EBRT [18, 19, 20]. In the ProtecT trial, Donovan et al. [21] analyzed PROMs for 1643 patients who underwent AS, operative treatment or radiation therapy, with a follow-up period of 72 months. The authors state that operative treatment is associated with a reduction in UI and sexual function scores, to a greater extent compared to EBRT, and that despite the variability of symptom scores in terms of improvement after 12 months, the difference between the mentioned groups remains during 72 months of follow-up. As in our study, the difference in UI scores in RP versus EBRT remains approximately the same during the follow-up period. The same authors reported that bowel scores were lower in the EBRT group, which is consistent with the results of our study.

Analyzing the effects of individual therapeutic modalities on the outcome of PCa treatment, it is worth mentioning that the recent meta-analysis by Cheng et al. [22] showed that the overall survival (OS) in RP is significantly higher compared to EBRT, with a similar cancer-specific survival, and that the risk of cancer-specific mortality is higher in EBRT. A recent systematic review by Greenberger et al. [23] on the effects of surgery, radiation, and ADT for the primary treatment of LPCa showed that there is still no strong evidence to favor any of these therapies in terms of overall mortality (OM) and cancer-specific mortality. In a study that using the International Prostate Symptom Score (IPSS) PROM instrument analyzed the impact of ORRP on postoperative voiding quality, ORRP was associated with a significant reduction in IPSS score and improvement in quality of life, over a 12-month follow-up period [24]. Hoffman et al. [20] conducted a prospective PROMs study for AS, surgery, PPB, EBRT or ADT, of 1386 men with LPCa, using the EPIC-26 instrument, with a five-year follow-up. In the sexual domain, there is a continuous decrease, both with RP and EBRT. Overall, the authors found no statistically significant differences in HRQoL between RP and EBRT, combined with ADT [20]. The UI score declines with RP until sixth month and recovers slightly afterwards, but is significantly lower than with EBRT during follow-up. Urinary symptoms were more pronounced with EBRT during the entire follow-up period. During the first year, the bowel score is slightly lower with EBRT compared to RP, but without a statistically significant difference. According to our results, this study, as well as the ProtecT trial, showed that RP significantly affects the reduction of urinary and sexual scores during the follow-up period, and that RP has the greatest negative effect on sexual scores [20, 21].

Our results in terms of sexual scores show a continuous trend of reduction during the follow-up period in both studied groups, at six and 12 months, after which a slight improvement is noticeable at 24 months. However, the overall reduction is statistically significant compared to baseline ($p < 0.001$).

Unlike the previously mentioned studies [20, 21], no statistically significant difference was found among the observed groups in our study, in any of the sexual score categories, at six, 12, and 24 months, which can be explained by a statistically significant difference in age at EBRT. Compared to the baseline, in our study group ORRP showed statistically significant variations in all urinary scores, during the entire follow-up period. The

incontinence score shows a significant decline at six months, followed by a statistically significant improvement that is most pronounced at 24 months. It is interesting that the urinary summary score shows variations, starting with a significant decrease in the sixth month, with a continuous statistically significant improvement over time, approaching the values from the baseline. This result is consonant with the results of most of other studies [20, 25].

In our study, the incontinence score was also significantly reduced in EBRT at six months, with an additional reduction at 12 months. Urinary function score decreases after treatment and maintains approximately the same values at six, 12, and 24 months. It is interesting that the increase in the urinary bother score and the UIO was recorded only in the 24th month. In this group, the urinary summary score was reduced at six and 12 months, but after 24 months it was increased. It should be noted that many patients from this group are on chronic drug therapy for lower urinary tract symptoms. During follow-up at six, 12, and 24 months, urinary summary, urinary function, urinary bother, UI and UIO, were lower in ORRP, showing that the negative effect of ORRP on urinary symptoms was more pronounced compared to EBRT, and this difference is statistically significant. However, the recovery of the same score in ORRP after 24 months in our patients may be due to the preserved muscle mass of the urethral rhabdosphincter (younger patients), with its good preservation during the performance of vesicourethral anastomosis. When it comes to bowel scores, both bowel function and bowel bother and bowel summary scores at ORRP show no variation during the follow-up period ($p > 0.01$). With EBRT, these scores progressively decrease statistically significantly and are the lowest in the 24th month. All three bowel scores are lower in EBRT compared to ORRP at six, 12, and 24 months, and this difference is statistically significant ($p > 0.05$).

In our study, the use of PROMs for assessing of the urinary, intestinal and sexual domains after ORRP or EBRT in LPCa, clearly established the set parameters, even their temporal variability in each of the set categories. Certain conclusions are relevant, such as that UI and sexual dysfunction are more prevalent in ORRP, and intestinal dysfunction in EBRT. However, since these PROMs are personalized instruments, the question of objectification and validation of certain conditions (e.g. personal interpretation of urinary complaints without urodynamic findings, etc.) can be raised, taking into account the adaptability of patients to side effects. Also, it is necessary to expand the profiles of PROMs towards psychometric aspects in the quantitative evaluation of the results, and in this respect the Consensus-based Standards for the Selection of Health Measurement Instruments methodology is promising [9, 26]. The issue of evaluating the results of multimodal treatment also arises. In this regard, it is necessary to conduct multi-institutional and prospective studies, as well as equalize inclusion criteria and research methodology in order to obtain data of a high level of coherence. For the synthesis and processing of data, it is necessary to expand the information network, based on the PIONEER Consortium [27].

CONCLUSION

In our study group, both ORRP and EBRT are associated with decline of sexual scores, while ORRP showed significant variations in all urinary scores, with more pronounced negative impact on urinary symptoms compared to EBRT, during the entire follow-up period. Bowel scores are lower in EBRT. Future research should include a more extensive consideration in terms of the psychometric domain of the PROM, which would greatly improve the synthesis and quantitative evaluation of the data.

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Отворена ретропубична радикална простатектомија наспрам спољашње зрачне терапије за локализовани карцином простате – исходи које пријављују болесници

Драгослав Башић[1,2], Александар Скакић[1,2], Милош Стевић[1,3], Александра Игњатовић[1,4], Жарко Мирковић[5], Иван Игњатовић[1,2], Јован Јанић[2], Андреј Вељковић[6], Љубинка Јанковић-Величковић[7], Јован Хаџи-Ђокић[8]

[1]Универзитет у Нишу, Медицински факултет, Ниш, Србија;
[2]Универзитетски клинички центар Ниш, Клиника за урологију, Ниш, Србија;
[3]Универзитетски клинички центар Ниш, Центар за нуклеарну медицину, Ниш, Србија;
[4]Универзитет у Нишу, Медицински факултет, Катедра за медицинску статистику и информатику, Ниш, Србија;
[5]Универзитетски клинички центар Ниш, Клиника за онкологију, Ниш, Србија;
[6]Универзитет у Нишу, Медицински факултет, Катедра за биохемију, Ниш, Србија;
[7]Универзитетски клинички центар Ниш, Центар за патологију и патолошку анатомију, Ниш, Србија;
[8]Српска академија наука и уметности, Београд, Србија

САЖЕТАК

Увод/Циљ Активне опције лечења локализованог рака простате укључују операцију и радиотерапију са андрогеном депривационом терапијом у одабраним случајевима, али све опције имају нежељене ефекте, углавном усмерене на уринарну, сексуалну и цревну функцију. Наша студија је имала циљ да процени и упореди мере исхода које су пријавили болесници након отворене ретропубичне радикалне простатектомије (ОРРП) или спољашње зрачне терапије (С3Т).

Методе У периоду од јуна 2019. до маја 2021. године, укупно 120 болесника са локализованим раком простате подвргнуто је активном лечењу, и то ОРРП – 60 болесника и С3Т – 60 болесника. За процену мера исхода које су пријавили болесници коришћен је валидирани упитник, композитна кратка форма са проширеним индексом рака простате (*EPIC*-26), кроз следеће домене: уринарни, цревни и сексуални. Болесници су попуњавали упитник на почетку и шест, 12 и 24 месеца после примарног лечења.

Резултати Сви резултати уринарног домена имају статистички значајну интеракцију између групе и времена. После шест, 12 и 24 месеца, сви уринарни резултати били су статистички значајно нижи у групи ОРРП. После 12 и 24 месеца, вредности цревног скора биле су статистички значајно ниже код болесника у групи С3Т. Сексуални резултати се мењају статистички значајно током периода праћења, без разлике међу групама ($p < 0,05$).

Закључак И ОРРП и С3Т повезане су са падом сексуалних скорова. ОРРП је показала значајне варијације у свим резултатима уринарног скора, са израженијим негативним утицајем на уринарне симптоме у поређењу са С3Т током читавог периода праћења. Резултати цревног скора нижи су код С3Т.

Кључне речи: локализовани карцином простате; отворена ретропубична радикална простатектомија; спољашња зрачна терапија; исходи пријављени од болесника

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The effects of complications and comorbidities on physical therapy duration in children with pneumonia

Biljana Među[1,2], Olivera Ćalović[3], Marija Karličić[1], Mišela Raus[1,2], Vladimir Radlović[1,2], Dejan Nikolić[1,2]

[1]University Children's Hospital, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[3]Dr. Dragiša Mišović – Dedinje Clinical Hospital Center, Children's Hospital for Lung Diseases and Tuberculosis, Belgrade, Serbia

SUMMARY

Introduction/Objective Physical therapy aims to improve airway clearance, breathing, and enhance gas exchange. It is widely used as an additional therapy in children with pneumonia. The aim of this study was to assess the effects of complications and comorbidities on physical therapy duration in children with pneumonia.

Methods We conducted a retrospective descriptive study including 40 children with pneumonia admitted to a tertiary children's hospital. Study participants were divided into two groups – a group with and without complications and a group with and without comorbidities. All children received physical therapy one time daily five days a week plus standard treatment for pneumonia. Physical therapy procedures that were applied were chest physical therapy and kinesiotherapy.

Results Chest physical therapy ($p < 0.001$) and kinesiotherapy ($p = 0.024$) were applied significantly longer in the group with complications versus those without complications. Chest physical therapy was applied significantly longer in the group with comorbidities versus the group without comorbidities ($p < 0.001$), while there was no difference regarding duration of kinesiotherapy in the group with and without comorbidities ($p = 0.239$).

Conclusion Our results show that the presence of complications and/or comorbidities significantly prolongs the duration of chest physical therapy in children with pneumonia.

Keywords: children; pneumonia; physical therapy

INTRODUCTION

Community-acquired pneumonia is an acute disease caused by an infection of the lung parenchyma acquired outside of a hospital setting [1]. Childhood pneumonia is still a significant clinical and public health problem and one of the leading causes of morbidity in children [2, 3]. Physical therapy is widely used as additional therapy in children with pneumonia. Currently in clinical practice different physical therapy techniques are available that aim to improve evacuation of inflammatory exudates and tracheobronchial secretions, remove airway obstruction, decrease airway resistance, improve gas exchange, and reverse pathological progression [4, 5]. However, strong scientific evidence is missing to support those beneficial effects in children with pneumonia and lack of data showing that physical therapy may contribute to patients' recovery. Authors of systematic review have concluded that there was insufficient evidence to make a clear recommendation for clinical practice and consequently reject or accept chest physical therapy as a standard treatment option in children with pneumonia [6]. However, to our knowledge, there are no studies investigating the effects of complications and comorbidities on physical therapy duration in this population. Therefore, the primary aim of this study was to assess the effects of complications and comorbidities on physical therapy duration in children with pneumonia. Additionally, we wanted to evaluate treatment outcome in study group regarding presence of complications and comorbidities.

METHODS

The study was based on a sample of 40 children (22 male and 18 female, mean age 34.5 ± 18.5 months, range 1 month to 10 years) hospitalized due to pneumonia. Pneumonia was defined as the presence of fever, acute respiratory symptoms (cough, tachypnoea, difficult breathing) or both, plus presence of new infiltrate on chest radiography or consolidation not attributable to some other etiology [7]. Exclusion criteria were severe concomitant disease (chronic pulmonary disease, cerebral palsy, immune deficiency), hemodynamic instability, chest drain, bone fragility or rib fractures [4]. The study was approved by the Ethics Committee of the University Children's Hospital in Belgrade (number 017 16/53).

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Correspondence to:
Biljana MEĐO
University of Belgrade
Faculty of Medicine
University Children's Hospital
Tiršova 10
Belgrade 11000, Serbia
medjo.biljana@gmail.com

Table 1. Duration of physical therapy procedures with regard to the presence of complications and pleural effusion

Tested parameters		Group with complications/ pleural effusion	Group without complications/ pleural effusion	p
Complications	Chest physical therapy (MV ± SD), days	11.82 ± 5.71	4.39 ± 1.64	< 0.001*
	Kinesiotherapy (MV ± SD), days	10.13 ± 6.26	4 ± 2.55	0.024*
Pleural effusion	Chest physical therapy (MV ± SD), days	10.83 ± 5.71	6.14 ± 5.6	< 0.001**
	Kinesiotherapy (MV ± SD), days	8.91 ± 5.49	8.4 ± 7.11	0.428*

MV – mean value; SD – standard deviation;
*Student's t-test;
**Mann–Whitney U test

Table 2. Duration of physical therapy procedures with regard to the presence of comorbidities

Tested parameters		Group with comorbidities	Group without comorbidities	p
Comorbidities	Chest physical therapy (MV ± SD), days	11.4 ± 6.1	6.27 ± 4.51	< 0.001*
	Kinesiotherapy (MV ± SD), days	9.7 ± 6.18	7.73 ± 6.26	0.239**

MV – mean value; SD – standard deviation;
*Mann–Whitney U test;
**Students t-test

Table 3. Duration of physical therapy procedures with regard to the presence of complications and comorbidities

Tested parameters		Group with complications and comorbidities	Group without complications and comorbidities	p
Complications and comorbidities	Chest physical therapy (MV ± SD), days	12.63 ± 6.21	4.19 ± 1.5	< 0.001*
	Kinesiotherapy (MV ± SD), days	10.75 ± 6.41	3 ± 1.73	0.038*

Out of 40 children with pneumonia, 17 had complications. The most common complications were pleural effusion in 12 children, empyema in three children, and necrotizing pneumonia in two children. Among 40 children with pneumonia, comorbidities were present in 10 children. Five children had congenital heart defects, two had repaired esophageal atresia with tracheoesophageal fistula, and one child had epilepsy, celiac disease, and Hirschsprung disease.

All the children received physical therapy one time daily with standard treatment for pneumonia (antibiotic therapy, fluid therapy, and oxygen, if needed, administered by the attending pediatrician) until discharge. Regarding physical therapy procedures, two modes were evaluated: chest physical therapy and kinesiotherapy. Each session of physical therapy was about 30 minutes and consisted of postural drainage, thoracic squeezing, chest percussion, vibration, cough stimulation, aspiration of secretions (if needed), and kinesiotherapy [4, 8]. The positions for postural drainage were directed by the chest radiograph to provide more effective drainage of secretions and exudates from the most affected areas [4, 8]. The decision to discharge from hospital was made by the attending pediatrician. Regarding treatment outcome two categories were assessed: discharge and prolonged hospitalization. Prolonged hospitalizations in this study were defined as those lasting 14 days or longer.

Statistical analysis

Results were presented as whole numbers (N) and percentages (%), while continuous values were presented as mean values (MV) with standard deviation (SD). Student's t-test and Mann–Whitney U test were used to compare continuous variables depending on the normality of distribution, and χ^2 test or Fishers exact test were used for categorical variables. Spearman rank correlation was used to measure the degree of association between the presence and number of complications or comorbidities and the duration of physical therapy. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

In the group of children with complications, chest physical therapy ($p < 0.001$) and kinesiotherapy ($p = 0.024$) were applied significantly longer compared to children without complications (Table 1). Moreover, it was shown that chest physical therapy was applied significantly longer in the group of children with pleural effusion compared to children without pleural effusion ($p < 0.001$), whereas there was no difference regarding the duration of kinesiotherapy in children with and without pleural effusion ($p = 0.428$) (Table 1).

In the group of children with comorbidities, chest physical therapy was applied significantly longer compared to children without comorbidities ($p < 0.001$) while there was no difference regarding the duration of kinesiotherapy in children with and without comorbidities ($p = 0.239$) (Table 2).

In addition, when we analyzed patients who had comorbidities and complications, we noticed that in those children both chest physical therapy ($p < 0.001$) and kinesiotherapy ($p = 0.038$) were applied significantly longer compared to children without comorbidities and complications (Table 3).

There was a statistically significant correlation between the presence and number of complications and the duration of chest physical therapy ($p < 0.001$) as well as with the duration of kinesiotherapy ($p < 0.001$) (Table 4). Furthermore, there was statistically significant correlation between the presence and number of comorbidities and the duration of chest physical therapy ($p < 0.001$), while

Table 4. Correlations between the duration of physical therapy modes and the presence and number of complications and comorbidities

Tested parameters		Duration of physical therapy	
		r	p
Presence and number of complications	Chest physical therapy	0.827	< 0.001
	Kinesiotherapy	0.673	< 0.001
Presence and number of comorbidities	Chest physical therapy	0.522	< 0.001
	Kinesiotherapy	0.274	0.229
Presence and number of complications and comorbidities	Chest physical therapy	0.781	< 0.001
	Kinesiotherapy	0.746	0.008

r – correlation coefficient

Table 5. Treatment outcome in tested patients regarding the presence of complications and comorbidities

Presence of complications and/or comorbidities	Treatment outcome	Patients		p
		N	(%)	
Complications				
Yes	Discharge	7	41.2	< 0.001*
	Prolonged hospitalization	10	58.8	
No	Discharge	23	100	
	Prolonged hospitalization	0	0	
Comorbidities				
Yes	Discharge	4	40	0.007**
	Prolonged hospitalization	6	60	
No	Discharge	26	86.7	
	Prolonged hospitalization	4	13.3	
Complications and comorbidities				
Yes	Discharge	2	25	< 0.001**
	Prolonged hospitalization	6	75	
No	Discharge	21	100	
	Prolonged hospitalization	0	0	

*χ^2;
**Fisher's exact test

no statistically significant correlation between the presence and number of comorbidities and the duration of kinesiotherapy (p = 0.229) was found (Table 4). Moreover, there was statistically significant correlation between the presence of comorbidities and complications and the duration of chest physical therapy (p < 0.001) as well as with the duration of kinesiotherapy (p = 0.008) (Table 4).

More than half of tested patients with complications had prolonged hospitalization (58.8%), while none of those without complications had prolonged hospitalization (0%). Regarding comorbidities, also more than half of children with comorbidities (60%) had prolonged hospitalization, while only 13.3% of those without comorbidities had prolonged hospitalization. Patients with complications and comorbidities had the highest proportion of prolonged hospitalization (75%), while none of those without complications and comorbidities had prolonged hospitalization (0%) (Table 5).

DISCUSSION

In this study we assessed the application of physiotherapy in children with pneumonia. There are very few studies of physical therapy in children with pneumonia and the results of those studies are controversial. A randomized trial from Brazil found that chest physical therapy as supplementary to standard treatment did not hasten the clinical resolution of children hospitalized with acute pneumonia and that physical therapy may prolong duration of coughing and rhonchi [9]. Another randomized study from Brazil demonstrated that the chest physical therapy had no beneficial effects in children hospitalized with community-acquired pneumonia [10]. In contrast, the authors of a more recent study from Egypt concluded that chest physical therapy showed significant improvements in children hospitalized with pneumonia. They reported that children treated with standard treatment for pneumonia and chest physical therapy had shorter time to clinical resolution and greater improvement in respiratory rate and arterial oxygen saturation compared to children treated with standard treatment for pneumonia alone, without chest physical therapy [11]. Moreover, the authors from Portugal studying adult outpatients with lower respiratory tract infections, recently found that adding respiratory physical therapy to the pharmacological treatment results in greater recovery of symptoms and function parameters [12]. Given the observations of above-mentioned studies, it should be pointed out that physical therapy particularly chest physical therapy in patients with pneumonia could lead to elimination and reduction of mucus in lung airways, thus affecting recovery and onset prevention and further deterioration of present complications [13].

Bearing in mind that pediatric population consists of very young infants up to the patients 18 years of age, modifications to physical therapy procedures are applied [13]. Susan and Hintz [14] pointed out that chest physical therapy used in infants was associated with improved oxygenation and secretion clearance and improvements in respiration and chest sound. Furthermore, Leelarungrayub et al. [15] reported that chest physical therapy possibly reduces oxidative stress and enhance oxygenation status in infants with pneumonia. These findings clearly demonstrate the importance of chest physical therapy in children, particularly infants with pneumonia.

According to the literature, there is still a lack of scientific evidence to make a clear, justified recommendation for the clinical practice, supporting or refusing physical therapy in children or adults with pneumonia. Authors of a recent systematic review on chest physical therapy in children with pneumonia emphasized that no reliable conclusions can be drawn concerning the use of chest physical therapy for children with pneumonia due to the small number of included trials with differing study characteristics and statistical presentation of data [16]. Moreover, a recently concluded systematic review stressed that current evidence was very uncertain about the beneficial effect of chest physical therapy in adults with pneumonia [17].

To our knowledge, this is the first study to present effects of complications and comorbidities on physical therapy duration in children with pneumonia. Our results showed that among children with pneumonia, those with additional complications had significantly longer chest physical therapy and kinesiotherapy than those without complications. Another important finding in our study is that we observed a statistically significant correlation between the presence and number of complications and the duration of chest physical therapy and duration of kinesiotherapy procedures. This is expected, since pneumonia with complications should be treated longer to gain improvements and the resolution of complications. Additionally, we found that children with complicated pneumonia more often had prolonged hospitalization, which is in agreement with data in literature [18, 19].

Furthermore, children with comorbidities, had significantly longer chest physical therapy versus those without comorbidities, while there was no difference regarding the duration of kinesiotherapy. Moreover, there was statistically significant correlation only between the presence and number of comorbidities and the duration of chest physical therapy. These findings demonstrate that the role of chest physical therapy is important in children with pneumonia and additional comorbidities, and this is further supported by the findings that comorbidities alone or with complications are significantly more frequent in the one with prolonged hospitalizations. In contrast, authors from Brazil did not find longer hospital stays in children with community-acquired pneumonia and comorbidities [20].

However, it should be considered that the results in our study might be influenced by the fact that the evaluated group of patients were presenting to a tertiary medical facility – a university children's hospital – therefore, patients with more severe infections may have been overrepresented, some of which could affect the production and elimination of secretions.

The present study has some limitations that should be considered in the analysis and interpretation of the results. Firstly, the study design included a retrospective collection of information. We did not have a control group due to ethical reasons, considering that respiratory physical therapy is often prescribed in children with pneumonia in our institution. In addition, the prolonged hospitalization was used as an endpoint in this study, although it is known that the decision about the duration of hospitalization varies among doctors and hospitals [10]. However, our study was conducted at a single institution; therefore, the doctor/facility profiles were unlikely to have biased length of hospitalization.

CONCLUSION

Our results suggest that the presence of complications and/or comorbidities significantly prolongs the duration of chest physical therapy and prolongs hospitalization in children with pneumonia.

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Утицај компликација и коморбидитета на трајање физикалне терапије код деце са пнеумонијом

Биљана Међо[1,2], Оливера Ћаловић[3], Марија Карличић[1], Мишела Раус[1,2], Владимир Радловић[1,2], Дејан Николић[1,2]

[1]Универзитетска дечја клиника, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија;
[3]Клиничко-болнички центар „Др Драгиша Мишовић – Дедиње", Дечја клиника за плућне болести и туберкулозу, Београд, Србија

САЖЕТАК

Увод/Циљ Физикална терапија има за циљ да побољша мукоцилијарни клиренс дисајних путева, дисање и размену гасова. Често се користи као додатна терапија у лечењу деце са пнеумонијом.

Циљ овог истраживања био је да се процени утицај компликација и коморбидитета на трајање физикалне терапије код деце са пнеумонијом.

Методе Ова ретроспективна дескриптивна студија је обухватила 40 деце са пнеумонијом која су лечена у терцијарној болници. Испитаници су били подељени у две групе – у групу са компликацијама и без компликација и у групу са коморбидитетом и без коморбидитета. Код све деце физикална терапија је примењивана једном дневно, пет дана у недељи уз стандардну терапију за лечење пнеумоније. Програм физикалне терапије је подразумевао респираторну рехабилитацију и кинезитерапију.

Резултати У групи испитаника са компликацијама у односу на испитанике без компликација значајно дуже су примењиване респираторна рехабилитација ($p < 0,001$) и кинезитерапија ($p = 0,024$). Такође, респираторна рехабилитација примењивана је значајно дуже у групи испитаника са коморбидитетима у односу на испитанике без коморбидитета ($p < 0,001$). Разлика у дужини кинезитерапије између ове две групе испитаника није утврђена ($p = 0,239$).

Закључак Наши резултати показују да присуство компликација и/или коморбидитета значајно повећава трајање респираторне рехабилитације код деце са пнеумонијом.

Кључне речи: деца; пнеумонија; физикална терапија

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Hepcidin as a biomarker of neonatal infections

Jelica Predojević-Samardžić[1,2], Dragana Malčić-Zanić[1,2], Želimir Erić[2], Gordana Guzijan[1,3], Sergej Samardžić[2]

[1]University of Banja Luka, Faculty of Medicine, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; [2]University Clinical Center of the Republic of Srpska, Banja Luka, Republic of Srpska, Bosnia and Herzegovina; [3]Institute for Transfusion Medicine of the Republic of Srpska, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

SUMMARY

Introduction/Objective Nonspecific clinical signs of neonatal infection dictate routinely determination of C-reactive protein (CRP) and procalcitonin levels in order to confirm the diagnosis. As hepcidin is an acute phase reactant, the aim of our study was to analyze its significance in diagnosis of neonatal infections.

Methods The prospective study included 71 term neonates, 37 with signs of infection in the absence of other pathological conditions and 34 healthy neonates. After standard bacteriological examination, at the time of diagnosis and after six days of antibiotic therapy, complete blood count, serum CRP, procalcitonin, and hepcidin were determined.

Results There was no difference in serum hepcidin levels between the control (55.17 ± 21.22 ng/ml) and the infection group (59.72 ± 59.7 ng/ml) on the first day. Hepcidin values in neonates with infection up to 72 hours were significantly lower (30.2 ng/ml, IQ: 25.9–39.9 ng/ml) than in older neonates (82.2 ng/ml, IQ: 39.7–128.1 ng/ml). In neonates up to 72 hours, after six days of antibiotics, the hepcidin values show a significant increase (36.68 ng/ml, IQ; 31.23–50.3 ng/ml). High hepcidin values (128.05 ng/ml, IQ: 60.95–201 ng/ml) were recorded in neonates with CRP over 100 mg/l.

Conclusion Our results shows that the determination of serum hepcidin as a marker of neonatal infection is not relevant in neonates up to 72 hours of life. After six days of antibiotic therapy, the neonates of this group reacted with an increase in hepcidin, while the parallel determined values of CRP and procalcitonin showed a significant decrease.

Keywords: neonates; infection; hepcidin; CRP; procalcitonin

INTRODUCTION

Despite progress in the diagnosis and treatment of infections, neonatal infections remain a global challenge in perinatal medicine and treatment of neonates [1].

Neonatal infections are defined as all infections occurring during the first 28 days of life. Due to the possibility of rapid progression and unfavorable outcome of infection in neonates, antibiotic therapy must be started before obtaining positive microbiological culture. Prompt diagnosis is one of the most delicate challenges in neonatology. Clinical (Tollner) and hematological (Rodwell) scoring systems, as well as determination of inflammatory biomarkers such as C-reactive protein (CRP) and procalcitonin (PCT) are of great importance in the diagnostic process [2, 3, 4].

It is known that the specificity of CRP and PCT in differentiating infectious from non-infectious causes of the inflammatory response is limited [5]. Also, unreliable values of CRP and PCT in premature neonates limits their diagnostic value [4]. In order to overcome these problems, it is necessary to find new biomarkers of infection that would enable well-timed diagnosis, monitor disease progression and evaluating the effect of treatment.

It has been proven that proinflammatory cytokines cause increased expression of hepcidin (a 25-amino acid peptide containing eight cysteine residues), which is now considered an acute phase reactant type II [6, 7]. Several studies have demonstrated the antibacterial activity of hepcidin-25 and hepcidin-20 against a large number of Gram-positive and Gram-negative bacteria [8, 9, 10]. The role of hepcidin in innate immunity is not only significant in infection – it can also have antitumor effects [7, 10, 11]. Hepcidin leads to the retention of iron in macrophages and decreases its absorption in the intestines, which results in hypoferremia and contributes to the host's defense against pathogens [7, 11, 12, 13]. Based on the present knowledge, it will be expected that neonates with infection would have significantly higher serum hepcidin levels than healthy ones.

The aim of our study was to examine the potential significance of hepcidin as a marker of neonatal infection, compared with the serum levels of CRP and procalcitonin as standard diagnostic markers.

METHODS

A prospective study was conducted at the Clinic for Children's Diseases and the Clinic for

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Correspondence to:
Dragana MALČIĆ-ZANIĆ
Childrens' Hospital
University Clinical Center of the Republic of Srpska
12 babies 2a
Banja Luka 78000
Republic of Srpska
Bosnia and Herzegovina
dragana.malcic@med.unibl.org

Gynecology and Obstetrics, University Clinical Center of the Republic of Srpska. The research was approved by the Ethics Committee of the University Clinical Centre of Banja Luka and was conducted in accordance with the principles of the Helsinki Declaration. For all neonates before inclusion in the study, the parents signed the informed consent for the child's participation. The study included 71 term neonates: 37 with clinical and laboratory signs of infection in the absence of other pathological conditions and 34 healthy neonates with matched demografic characteristics.

In addition to the clinical evaluation (collection of anamnestic data on risk factors for neonatal infections, clinical examination, monitoring of the course of treatment and outcome), capillary and venous blood was taken from all the subjects.

The clinical examination consisted of an assessment of the child's general condition, assessment of skin color, vital parameters, body temperature, physical findings by organs and systems, and determination of the Tollner sepsis score [2].

In laboratory analyses, in addition to the complete blood count, peripheral blood smear, C-reactive protein, procalcitonin, hepcidin concentration were performed for all subjects. The hematological sepsis score according to Rodwell was determined for all neonates [3].

In neonates with infection, blood was taken at two time points: at the time of clinical diagnosis of infection and blood collection for culture, and after six days of antibiotic therapy, as part of venous blood collection for routine tests.

In both time points, 3 ml of peripheral blood was drawn into a tube without anticoagulant. Upon separating the serum by centrifugating the samples at 3000 × g, part of the serum was kept frozen at -70°C until the time of measurement of hepcidin levels. Other parameters were measured immediately.

The complete blood count was obtained by routine methods on Advia 2120 (Siemens Healthineers AG, Forchheim, Germany). Serum concentration of C-reactive protein and prokalcitonin were measured by routine methods on Roche/Hitachi Cobas 6000 using commercial Roche's kits. Values of CRP below 10 mg/l were considered physiological [14]. According to personal experiance values of 10–40 mg/l were considered a low risk for bacterial infection, 40–100 mg/l were considered a moderate risk, and values above 100 mg/l were considered a high risk for bacterial infection.

Concentrations of procalcitonin below 0.5 ng/mL were considered physiological. Values 0.5–2 ng/ml defined a moderate risk for sepsis, while values above 2 ng/ml represented a high risk for sepsis.

The level of hepcidin in the serum was measured by the by enzyme-linked immunosorbent assay intended strictly for research (DRG Hepcidin 25 ELISA; EIA 5258) according to the manufacturer's instructions on an open system device (BP 2000, Siemens). The results were expressesd in ng/ml.

Data distribution was tested using the Kolmogorov–Smirnov test. Depending on the type of variables and the normality of distribution, Student's t-test and Mann–Whitney U test were used to test data differences between two independent samples. Categorical variables were tested by the χ^2 test. Wilcoxon signed-rank test and Student's t-test for dependent data were used to test changes between two repeated observations in the same group. Data are shown as mean ± standard deviation for normally distributed variables. The median for independent data or the median of difference for dependent data with interquartile range were presented for non-normally distributed variables. Relative or absolute frequencies are shown for categorical variables.

Statistical analysis and presentation of results were performed using Statistical Package for Social Sciences – SPSS Statistics for Windows, Version 17.0 (SPSS Inc., Chicago, IL, USA). A p-value < 0.05 was considered statistically significant.

RESULTS

The study included 71 neonates, divided into two groups: a control group of 34 healthy neonates and a group of 37 neonates with signs of infection and no other pathological conditions. The groups were similar in sex, age, gestational age, birth weight, birth length, and Apgar score (Table 1).

Table 1. Demographic characteristics of the study population

Parameter		Experimental group	Control group	p
Sex N (%)	Male	20 (54.05)	19 (55.88)	0.877*
	Female	17 (45.95)	15 (44.12)	
Age on admission (days) (median; IQ)		2 (2–5)	3 (3–4)	0.293**
Gestational age (days) (median; IQ)		279 (274–286)	279 (271–281)	0.185**
Apgar score 1st minute (median; IQ)		9 (9–9)	9 (9–9)	0.098**
Apgar score 5th minute (median; IQ)		10 (10–10)	10 (10–10)	0.054**
Birth weight (grams) (mean ± SD; 95% CI)		3637.84 ± 392.34 (3511.42–3764.26)	3605.88 ± 338.5 (3492.1–2790.66)	0.716***
Birth length (cm) (median; IQ)		53 (52–55)	53 (53–55)	0.480**

*Pearson's χ2 test;
**Mann–Whitney U test;
***Student's t-test

The Tollner sepsis score was positive in 14 (37.84%) neonates of the infection group, while score was negative in all neonates of the control group. Rodwell score was positive in 18 (48.65%) neonates in the infection group on the first day, while all neonates in the control group had a negative Rodwell score. The stated difference between the neonates of the control and infection group was statistically significant ($p < 0.001$).

Laboratory findings of our patients are shown in Table 2. The mean value of hepcidin in neonates with infection was 59.72 ± 59.70 ng/ml (95% CI: 40.48–8.96 ng/ml) and no significant differences in this parameter were observed compared to the control group (55.17 ± 21.22 ng/ml; 95% CI: 48.04–2.3 ng/ml).

Table 2. Laboratory characteristics of the study population

Parameter	Group	N	Mean ± SD (95% CI)	Minimum–maximum	Median (interquartile interval)	p
Erythrocytes ($\times 10^{12}$)	Control	34	4.68 ± 0.55 (4.5–4.86)	3.63–6.55	4.7 (4.38–4.95)	0.155*
	Experimental	37	4.51 ± 0.46 (4.36–4.66)	3.63–5.65	4.59 (4.22–4.71)	
Hemoglobin (g/l)	Control	34	164.79 ± 14.15 (159.43–170.16)	144–218	163.5 (149–173)	**0.025***
	Experimental	37	156.32 ± 15.18 (151.43–161.21)	122–193	158 (145–162)	
Hematocrit (l/l)	Control	34	0.49 ± 0.06 (0.47–0.51)	0.37–0.65	0.49 (0.46–0.52)	0.164*
	Experimental	37	0.47 ± 0.05 (0.43–0.49)	0.37–0.55	0.48 (0.45–0.5)	
Thrombocytes ($\times 10^{9}$)	Control	34	330.34 ± 6.843 (307.23–353.24)	226–493	321.5 (284–347)	0.613**
	Experimental	37	309.86 ± 91.48 (280.39–339.34)	127–478	321 (253–363)	
Leukocytes ($\times 10^{9}$)	Control	34	14.41 ± 4.8 (12.79–16.02)	5.66–26	14 (11.39–16.2)	0.102*
	Experimental	37	16.56 ± 6.02 (14.62–18.5)	6.01–29.32	15.99 (12.64–18.93)	
CRP (mg/l)	Control	34	3 ± 1.86 (2.38–3.63)	0.4–7.6	2.5 (1.8–3.9)	**< 0.001****
	Experimental	37	80.11 ± 44.1 (65.9–94.32)	29.1–241	65.5 (52.8–96.7)	
PCT (ng/ml)	Control	34	0.43 ± 0.3 (0.33–0.53)	0.05–0.97	0.34 (0.15–0.7)	**< 0.001****
	Experimental	37	41.44 ± 76.34 (16.84–66.83)	0.24–408.65	15.83 (3.23–35.86)	
Hepcidin (ng/ml)	Control	34	55.17 ± 21.22 (48.04–2.3)	17.99–13.3	57.07 (36.8–1.3)	0.061*
	Experimental	37	59.72 ± 59.7 (40.48–8.96)	86.51–252.1	34.79 (26.67–0.6)	

CRP – C-reactive protein; PCT – procalcitonin;
*Student's t-test;
**Mann–Whitney U test

Table 3. Hepcidin value in the group of neonates with infection

Group	Parameter	n	Hepcidin (ng/ml)			p
			Mean ± SD (95% CI)	Minimum–Maximum	Median (interquartile interval)	
All infections	1st day	37	59.72 ± 59.7 (40.48–78.96)	86.51–252.1	34.79 (26.67–70.6)	0.428*
	6th day	37	44.72 ± 22.04 (37.62–51.82)	9.1–105	36.73 (30.39–56.61)	
Infections 1st day	early onset infections	24	40.07 ± 43.5 (22.67–57.47)	13.30–235	30.19 (25.9–39.88)	**0.012****
	late onset infections	13	95.99 ± 69.78 (58.06–133.92)	19.54–252.1	82.20 (39.7–128.1)	
Infections 6th day	early onset infections	24	44.05 ± 19.34 (36.32–51.79)	25.12–105	36.68 (31.23–50.3)	1.000**
	late onset infections	13	45.94 ± 27.16 (31.18–60.7)	9.1–86.68	44.88 (27.16–66.2)	
Early onset infections	1st day	24	40.07 ± 43.5 (22.67–57.47)	13.3–235	30.19 (25.9–39.88)	**0.040***
	6th day	24	44.05 ± 19.34 (36.32–51.79)	25.12–105	36.68 (31.23–50.3)	
Late onset infections	1st day	13	95.99 ± 69.78 (58.06–133.92)	19.54–252.1	82.2 (39.7–128.1)	**0.005***
	6th day	13	45.94 ± 27.16 (31.18–60.7)	9.1–86.68	44.88 (27.16–66.2)	

*Wilcoxon W test;
**Mann–Whitney U test

In the group of neonates with infection overall, regardless of whether they were up to 72 hours old (early infections) or older (late infections), no statistically significant differences were observed in hepcidin values obtained on the first day (34.79 ng/ml; IQ: 26.67–70.60 ng/ml) compared to the sixth day (36.73 ng/ml; IQ: 30.39–56.61 ng/ml). The values obtained in the group of neonates with early infections (median 30.2 ng/ml, IQ: 25.9–39.9 ng/ml), were significantly lower compared to the values in neonates with late infections (82.2 ng/ml, IQ: 39.7–128.1 ng/ml). In neonates with early infections, hepcidin values on the first day (median 30.19 ng/ml, IQ: 25.9–39.88 ng/ml) were significantly lower than on the sixth day (36.68 ng/ml, IQ: 31.23–50.3 ng/ml). In neonates with late infections, hepcidin values on the first day (median 82.20 ng/ml, IQ: 39.70–128.10 ng/ml) were significantly higher than on the sixth day (median 44.88 ng/ml, IQ: 27.16–66.2 ng/ml) (Table 3).

In early infections, a slight increase in hepcidin values from the first to the sixth day (9.21 ng/ml; IQ: 1.12–22.03 ng/ml) is noticeable, while in late infections, there was a marked decrease in hepcidin values from the first to the sixth day (52.2 ng/ml, IQ: 10.44–69.95 ng/ml). This difference between early and late infections was significant (p = 0.001).

Our results showed statistically higher values of hepcidin (median 128.05 ng/ml, IQ: 60.95–201 ng/ml) in the group of neonates with CRP values over 100 mg/l (high risk of sepsis) compared to neonates with moderate (median 32.68 ng/ml, IQ: 24.69–43 ng/ml) and low (median 28 ng/ml, IQ: 27.14–29.56 ng/ml) risk of sepsis according to CRP (p = 0.012).

DISCUSSION

Our study included neonates born after 37 weeks of gestation, average postnatal age 4.27 days in the group of neonates with infection, or 3.21 days in the control group of healthy neonates. Premature babies were not tested, due to the underdevelopment of their immune system [15, 16].

Although positive culture is the "golden standard" in the diagnosis of infection, the percentage of positive culture results in different studies ranges 8–73% [17]. In our study, a positive blood culture was obtained in 10.72% of neonates. The frequency of positive urine cultures was 29.73%, and there was only one case with a positive cerebrospinal fluid culture (data not shown).

Prompt antibiotic therapy is required in order to prevent the development of sepsis, but time required to obtain culture results is at least 48 hours [18]. Due to the nonspecificity of clinical signs and long time to microbiological results, acute phase reactants have great importance in therapeutic decision. One widely used biomarker of neonatal infection is CRP. According to CRP values, all neonates in the control group were without signs of inflammation, while the mean level of CRP in the group of neonates with infection was 65.5 mg/l. The mean level of procalcitonin in the group of neonates with infection was 41.44 ± 76.34 ng/ml. In addition to these standard parameters of systemic inflammatory response that are used in clinical practice, we determined the values of hepcidin, an acute phase reactant synthesized in the infection.

Studies in children have not provided a uniform reference range of hepcidin values. The median (interquartile range) of hepcidin in the plasma of boys is 21.89 ng/mL (16.5–51.7 ng/ml), and that of girls is 21.95 ng/ml (19.2–47.7 ng/ml) [19]. The levels of hepcidin in cord blood of term neonates (19.40 ± 4.40 ng/ml) were similar to the values in preterm neonates (20.9 ± 13.8 ng/ml) [20]. In the study by Kulik-Rechberger et al. [21], hepcidin values of healthy neonates on the third day of life were 66.79 ± 22.85 ng/ml. The median value of hepcidin in the serum of preterm neonates who required erythrocyte transfusions, in the study by Muller et al. [22] was 52.4 ng/ml, about 30% lower than the hepcidin values detected in the umbilical blood of term neonates in previous studies [23]. Lower hepcidin values in premature neonates are thought to be the result of reduced iron reserves due to a shorter gestation [22].

In their study, Koukoulas et al. [24] found significantly increased hepcidin levels in patients with bacteremia on day 0 and day 7, compared to healthy controls, and a significant reduction of serum hepcidin after a seven-day treatment. In a prospective study, Olinder et al. [25] have shown that hepcidin values are significantly higher in septic than in non-septic patients.

In the study by Sherbiny et al. [26], hepcidin values in the serum of premature neonates with late-onset sepsis (288 ± 81.3 ng/ml) were significantly higher compared to the control group (66 ± 12 ng/ml). They have also shown a significant decrease of hepcidin (to 98.3 ± 31 ng/ml) after a seven-day antibiotic therapy to 98.3 ± 31 ng/ml [26].

In their research, Wu et al. [27] found four times higher hepcidin values in neonates with late-onset sepsis compared to healthy neonates (26.8–67.7 ng/ml) and neonates with infection who did not develop sepsis (5.3–89.8 ng/ml). The results of the study by Cizmeci et al. [28] show elevated values of hepcidin in the umbilical cord blood of neonates who developed early neonatal sepsis. Similar results were obtained by Motalib et al. [5] among neonates with early neonatal sepsis. Wu et al. [27] and Motalib et al. [5] have also proven a significant correlation between hepcidin and CRP. In the study by Delaby et al. [29] in adult patients, subjects with CRP below 10 mg/dl had a mean hepcidin values of 4.64 ng/ml, while those whose CRP level was higher than 10 mg/dl had a mean hepcidin level of 55.85 ng/ml.

In our study, hepcidin values in neonates with infection on the first day were not significantly different from hepcidin values in healthy neonates. However, hepcidin values in neonates with late infections were significantly higher compared to neonates with early infections ($p = 0.012$). In the group of neonates with early infections, hepcidin values on the sixth day were significantly higher compared to the values on the first day ($p = 0.040$). In contrast to this, in the group of neonates with late infections, the values obtained on the sixth day were significantly lower compared to the first day ($p=0.005$). The values of hepcidin in neonates with a high risk of sepsis according to the CRP value were 131.52 ± 84.37 ng/ml, which was higher than the values in the low- and moderate-risk group, indicating that the severity and intensity of inflammatory response in severe bacterial infections lead to a significant increase in hepcidin, which is in line with other studies [5, 27, 29].

It remains questionable whether the observed differences in hepcidin values are a consequence of maturation and a better response to various stimuli with increasing postnatal age, or a consequence of the longer time from the onset of infection to blood sampling for analysis, considering the fact that in our study the "older" neonates were hospitalized from home, while neonates with early infections had not been discharged and the signs of infection were recognized earlier. According to our results, early infection (from the first to the sixth day) is associated with an increase in hepcidin levels, while late infections are followed by a decrease in hepcidin. Thus, the relationship between observed hepcidin levels and the time of infection recognition and blood sampling in our study appear to be in agreement with the expected kinetics of hepcidin synthesis during the inflammatory response.

Even though our study has certain limitations, due to the small number of patients, the diversity of severity and time of infection onset, based on the results obtained, we summarized that hepcidin values are dependent on postnatal age. The neonatal period is also characterized by numerous changes in hematopoiesis and iron metabolism, in which hepcidin plays a significant role.

CONCLUSION

According to our results, serum hepcidin may not be a relevant marker of neonatal infection in the first 72 hours of life. After six days of antibiotic therapy, all investigated neonates had increased hepcidin levels, while their CRP and procalcitonin levels were decreased. Despite the significant increase in hepcidin found in neonates with CRP above 100 mg/l, we conclude that hepcidin has no

significant advantages as a tool to ascertain neonatal infection compared to CRP and procalcitonin and could be used only as an additional biomarker of serious neonatal infections. Additional studies of hepcidin level alterations are, however, clearly warranted, primarily in the healthy neonates, and also under pathological conditions.

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Хепцидин као биомаркер неонаталних инфекција

Јелица Предојевић-Самарџић[1,2], Драгана Малчић-Занић[1,2], Желимир Ерић[2], Гордана Гузијан[1,3], Сергеј Самарџић[2]

[1]Универзитет у Бањој Луци, Медицински факултет, Бања Лука, Република Српска, Босна и Херцеговина;
[2]Универзитетски клинички центар Републике Српске, Бања Лука, Република Српска, Босна и Херцеговина;
[3]Завод за трансфузијску медицину Републике Српске, Бања Лука, Република Српска, Босна и Херцеговина

САЖЕТАК

Увод/Циљ Неспецифичност клиничких знакова инфекције код новорођенчади налаже рутинско одређивање серумског нивоа Ц-реактивног протеина (ЦРП) и прокалцитонина у циљу потврде дијагнозе. Будући да је хепцидин реактант акутне фазе запаљења, циљ нашег рада је био испитати значај одређивања хепцидина у дијагностици неонаталних инфекција.

Методе Проспективним студијом обухваћено је 71 терминско новорођенче, 37 са клиничким и лабораторијским знацима инфекције без других патолошких стања и 34 здрава новорођенчета. После стандардне бактериолошке обраде, у време дијагнозе и након шест дана антибиотске терапије одређиване су вредности параметара у комплетној крвној слици, серумских нивоа ЦРП-а, прокалцитонина и хепцидина.

Резултати Није утврђена разлика у серумској вредности хепцидина између контролне групе (55,17 ± 21,22 *ng/ml*) и групе новорођенчади са инфекцијом (59,72 ± 59,7 *ng/ml*) у првом дану. Вредности хепцидина код новорођенчади са инфекцијом унутар 72 сата (30,2 *ng/ml*, *IQ*: 25,9–39,9 *ng/ml*) статистички су значајно ниже у односу на вредности новорођенчади старије од 72 сата (82,20 *ng/ml*, *IQ*: 39,7–128,1 *ng/ml*). Након шест дана антибиотске терапије вредности хепцидина код новорођенчади са инфекцијом узраста до 72 сата показују статистички значајан раст (36,68 *ng/ml*, *IQ*: 31,23–50,3 *ng/ml*). Високе вредности хепцидина (128,05 *ng/ml*; *IQ*: 60,95–201 *ng/ml*) забележене су само код новорођенчади са ЦРП-ом преко 100 *mg/l*.

Закључак Према резултатима нашег истраживања, одређивање серумског хепцидина као маркера неонаталне инфекције није релевантно код новорођенчади са инфекцијом до 72 сата живота. Након шест дана антибиотске терапије новорођенчад ове групе су реаговала порастом хепцидина, при чему су паралелно одређиване вредности ЦРП-а и прокалцитонина показале значајан пад.

Кључне речи: новорођенчад; инфекције; хепцидин; ЦРП; прокалцитонин

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Antioxidant and free radicals species in the aqueous humor of patients with age-related cataract

Stefan Brunet[1,2], Nikola Babić[1,2], Sofija Davidović[1,2], Aleksandar Miljković[1,2], Nikola Knezi[2], Vladimir Čanadanović[1,2,3]

[1]University Clinical Center of Vojvodina, Eye Clinic, Novi Sad, Serbia;
[2]University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia;
[3]Academy of Medical Science of the Serbian Medical Society, Beograd, Serbia

SUMMARY

Introduction/Objective Age-related cataract is a significant cause of visual impairment worldwide. Oxidative damage and the effects of free radical species are considered important in the etiopathogenesis of cataracts.

The aim of this study was to evaluate the antioxidative capacity and oxidative stress in the aqueous humor (AH) according to age and cataracts maturity.

Methods Clinical and biochemical researches were carried out in 55 patients with age-related cataract. According to the cataract maturity, patients were classified into incipient (cortical – group C, 18 patients; nuclear – group N, 20 patients; mature – group M, 17 patients). In order to evaluate the impact of age patients within each group were divided into Group I (65–69 years) and Group II (70 ≥ years). The antioxidant activity of AH was measured by the reduction power method and the activity of glutathione peroxidase (GPx) spectrophotometrically. Changes in the concentrations of hydroxyl and ascorbyl radicals were detected by electron spin resonance spectroscopy.

Results Both reduction power and GPx activity were significantly ($p < 0.001$) reduced in group N compared to group C and in group M compared to group N. Concentrations of hydroxyl (29.45 ± 1.01% in group C, 38.12 ± 1.29% in group N, and 74.14 ± 2.52% in group M) and ascorbyl radicals (26.12 ± 0.89% in group C, 41.15 ± 1.39% in group N, and 83.56 ± 2.84% in group M) increased significantly ($p < 0.05$) with progression of age-related cataract. Significant negative correlation ($r = -0.759$, $p < 0.05$) was determined between concentrations of HO• and content of GPx.

Conclusion Our research proved that the level of oxidative stress in the AH is significantly affected during aging and cataract progression, The obtained data support the hypothesis that during aging, depending on the maturity of the cataract, the antioxidant capacity in the AH decreases due to an increase in the concentration of reactive HO•.

Keywords: cataract; antioxidant enzyme; hydroxyl radical; ascorbyl radical

INTRODUCTION

Age-related cataract is a common cause of visual impairment which can significantly reduce patient's quality of life. As the world's population ages, an increase in the number of patients is expected. Knowing these facts, it is not surprising that there is large number of researches aimed at determining the cause and prevention of this disease [1]. The pathophysiology of age-related cataract is complex and still not fully understood. Several risk factors such as diabetes, malnutrition, diarrhea, poverty, sunlight, smoking, hypertension, and renal failure are associated with cataract formation [2]. Various studies have gradually confirmed that reactive oxidative species (ROS) play the most important role in the etiology of cataract formation. Opacification of the lens may be initiated by photochemically or non-photochemically oxidative damage [2]. The present hypothesis considers oxidative stress (OxS) as an important factor which can damage the crystalline proteins, lipids, polysaccharides, and nucleic acids during cataractogenesis [3].

By the nature of their functioning all aerobic organisms are continuously exposed to oxidants, such as free radicals (superoxide anion, hydroxyl, alkyl, peroxyl) and non-radical species (hydrogen-peroxide, ozone, singlet oxygen, organic peroxides). ROS levels are normally controlled by intracellular antioxidant defense mechanisms that include endogenous antioxidants such as enzyme systems [superoxide dismutase, catalase, glutathione peroxidase (GPx)], uric acid, bilirubin, glutathione, coenzyme Q10. Also, the exogenous antioxidants which include vitamin C, vitamin E, carotenoids, polyphenolic compounds participate in the stabilization and transformation of ROS in the secondary level of protection [4]. Unfortunately, with aging oxidative damage increases, antioxidant capacity decreases in the lens and in the aqueous humor (AH) and the efficiency of reparative systems become impaired. Such an imbalance in the organism is called OxS, which is the cause or accompanying factor in the pathology of many diseases [5].

The secretion of AH and the regulation of its conventional and non-conventional pathway are

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Correspondence to:
Vladimir ČANADANOVIĆ
University Clinical Center of Vojvodina
Eye Clinic
Hajduk Veljkova 1–9
21000 Novi Sad
Serbia
vladimir.canadanovic@mf.uns.ac.rs

physiologically important processes for normal eye function and their antioxidant capacity reflects the degree of OxS in the surrounding tissues [6]. Understanding the mechanisms of cataractogenesis should bring a better therapy.

Conditions leading the excess hydrogen-peroxide (H_2O_2) in AH may precipitate oxidative damage and cataract formation. The oxidizing agent, H_2O_2 is present in AH at concentration of approximately 20–30 μmol and it is reported to be raised (up to 660 μmol) in patient with cataract. Higher than normal levels of H_2O_2 production in the lens and/or AH could be via intraventricular, autoxidation of ascorbic acid, GSH and 3-hydroxykynurenine. Protein modifications linked with cataract could be the result of a reaction of crystalline lens, with the hydroxyl radicals (HO•), which derive from H_2O_2 through the transition-metal ion catalyzed Fenton reactions [7].

Several studies point out the possibility that one of the main functions of high concentration of ascorbic acid (vitamin C) in AH is to protect the lens and other surrounding tissues against the OxS induced by free radicals. In addition, large concentrations of ascorbic acid in the AH appear to provide significant protection against oxidative insult, and this possibly explains the occurrence of a high concentration of ascorbic acid in the AH [8]. Deprotonated form of ascorbic acid (ascorbate) forms covalent bonds with the crystalline lens, which reduces protein solubility [9]. But in the presence of ROS, particularly H_2O_2 or HO•, in the first and second one-oxidative reactions of ascorbic acid, ascorbyl radical (ASC•) and then dehydroascorbic acid it could be formed [10]. These reactions occur during increased OxS when the mechanism for maintaining the reduced form of ascorbic acid is compressed.

The difficulty in studying the role of free radicals in human cataract formation is the inability to measure directly these reactive species in the lens or AH *in vivo*. Electron spin resonance (ESR) spectroscopy provides a unique method to examine directly free radicals and it gives information about the concentration and structure of the radical centre's surroundings. Owing to the unpaired electron in the outer orbital, free radicals are paramagnetic species and when present in sufficient quantity, are detectable and measurable by ESR spectroscopy [11, 12]. Kinetic measurements and analysis the change in parameters of ESR spectra (line shape, linewidth, line intensity and *g*-factor) reflect important data about the reactions of free radicals in the AH due to the fact the epithelial surface of the lens is in contact with the aqueous fluid.

The purpose of this paper is to analyze parameters of OxS by testing the redox power (RP) and enzyme antioxidant power and formations of ascorbyl and HO• in the AH of patients with different age and maturity of age-related cataract.

METHODS

Chemicals and reagents

All chemicals and solvents were of the highest analytical grade.5,5-dimethyl-1-pyroline-N-oxide (DMPO), Trolox, potassium ferricyanide and trichloroacetic acid were purchased from Sigma Chemical Co. (Sigma-Aldrich, St Louis, MO, USA). Ferric chloride was obtained from J.T. Baker (Avantor Performance Materials B.V., Deventer, the Natherlands) and sodium nitrite from LACH-NER (Lach-Ner, Ltd., Neratovice, the Czech Republic). Total GPx assay kit was from Helvetica Health Care Sàrl, (Helvetica Health Care Sàrl, Geneva, Switzerland).

Patients

Clinical and biochemical researches were carried out in 55 patients (P1–P55) with age-related cataract. According to the cataract maturity patients were classified into incipient (cortical – group C, 18 patients, nuclear – group N, 20 patients, and mature – group M, 17 patients). In order to evaluate the impact of age patients within each group were divided into Group I (65–69 years) and Group II (70 years).

Patients with other ophthalmic (glaucoma, uveitis, retinal diseases, etc.) and systemic (diabetes, hyperlipemia, immunological etc.) diseases that might have influence on OxS were excluded.

Sample collection

The samples of the AH for analysis were taken immediately before the start of surgical procedure (phacoemulsification with intraocular lens implantation). All operations were performed according to the principles of sepsis and antisepsis. Through lateral limbal paracentesis from the space of the anterior chamber 0.15–0.20 ml, of AH was aspirated using a Gliss Wells cannula of 20 G. The amount of AH taken was compensated with isotonic Ringer-lactate solution and the surgical procedure was continued as usual.

Biochemical analysis of reduction power

RP was determined by the method of adapted for a 96-well microtiter plate [13]. Eppendorf tubes contained 75 μl of sample solution or 75 μl of extractant (blank test), 75 μl of Na-phosphate buffer, pH 6,6, and 75 μl of 1% potassium ferricyanide. Incubation was performed at 50°C and then 75 μl of 10% trichloroacetic acid was added. After centrifugation, 50 μl of distilled water and 10 μl of 0.1% ferric chloride were added to 50 μl of carefully separated supernatant. The absorbance of the samples was measured at a wavelength of 700 nm. A calibration curve was constructed with Trolox, and the results were expressed as mmol Trolox equivalents per ml of sample (mmolTE/ml).

Glutathione peroxidase activity assay

The activity of GPx was determined spectrophotometrically (UV-1800 spectrophotometer, Shimadzu, Kyoto, Japan). Total GPx assay kit provides a method of quantifying the activity of GPx (U/ml) [14]. The oxidation of NADPH to $NADP^+$ is monitored by a decrease in absorbance at 340 nm.

Electron spin resonance detection of reactive hydroxyl radicals

Based on the fact that HO• formed in Fenton's model system have a short lifetime (< 1 ms) and low concentration (< 10^{-7} M), ESR spectroscopy is combined with the "spin-trapping" method. This technique involves the addition-type reaction of a short-lived radical with a paramagnetic compound (spin-trap) to form a long-lived free radical product (spin-adduct), which can then be studied using ESR. In this work, DMPO was used as a spin trap, and the concentration of the resulting 5,5 Dimethyl 1 Pyrroline 1 Oxide (DMPO–OH) is equivalent to the concentration of HO• [12]. The system consisting of: 500 μmol H_2O_2, 75 μmol $FeCl_2$, 100 mmol DMPO (control sample). Data refer to the ESR signal intensity of DMPO–OH detected in the control sample defined as 1 (100%).

The influence of AH on the amounts of HO• trapped by DMPO was studied by adding 20 μl AH to the control system. ESR spectra were recorded with the following spectrometer settings: modulation amplitude 0.512 G, x-band frequency 9.64 GHz, receiver gain 1×10^4, center field 3440.00 G, sweep width 100.00 G, time constant 81.92 ms, conversion time 163.84 ms, power 20 mW.

The relative intensity of HO• ($RI_{•HO}$) value of the samples was defined as:

$$RI_{•HO} = 100 \times (h_x - h_0) / h_0 \ [\%].$$

There h_0 and h_x are the height of the second peak in the ESR spectrum of DMPO–OH spin-adduct of the control sample and the probe (control sample with AH), respectively.

Electron spin resonance detection of ascorbyl radicals

ASC• in AH were directly measured by ESR spectrometer with the same spectrometer settings adjusted for the determination of HO•.

Statistical analysis

All analysis were run in triplicate and were expressed as means ± standard deviation (SD). Statistical analyses were carried out using Origin Pro 8.0 software (OriginLab Corporation, Northampton, MA, USA). Significant differences were calculated by ANOVA and Tukey's test ($p < 0.05$).

We certify that institutional regulations concerning the ethical use of human volunteers were followed during this research. This study was approved by the Human Subjects Committee of the University of Novi Sad (00-209, November 26, 2021) and it adheres to the principles of the Helsinki Declaration. Written informed consent was obtained from all participants.

RESULTS

AH samples were obtained from 55 patients (P1–P55). The mean age in Group I was 67.41 ± 2.89, and 78.23 ± 3.42 in group II. There was no significant difference in gender and cataract type between Group I (nine cortical patients, 10 nuclear patients, and eight mature patients) and Group II (nine cortical patients, 10 nuclear patients, and nine mature patients).

Among our patients, a significantly ($p < 0.001$) higher concentration of RP was measured in AH of younger patients (Group I) with incipient cataract (C group 1.81 ± 0.07 mmolTE/ml; N group 0.76 ± 0.04 mmolTE/ml) compared to the mature (0.32 ± 0.08 mmolTE/ml) cataract. In elderly patients (Group II) the antioxidant status of AH decreases, what is the consequence that RP value also decreases in patients with incipient (C group 1.02 ± 0.04 mmolTE/ml; N group 0.66 ± 0.03 mmolTE/ml) and especially in those with mature cataract (0.15 ± 0.01 mmolTE/ml).

Analyzing the GPx activity in AH of patients with cataract, our results showed that the GPx activity was significantly reduced in the AH of patients with nuclear as compared to the cortical cataract. Testing the difference, depending on the maturity degree of the cataract, a significantly lower activity of GPx were measured in the group of patients with mature cataract in relation to the nuclear cataract group. The mean value of GPx in AH of patients in the Group II significant decreased on 2.81 ± 0.13 U/ml in C, 1.72 ± 0.06 U/ml in N and 0.98 ± 0.03 U/ml in M group, respectively, when compared with that of the patients from Group I in all cataract maturity (Table 1).

Mean values of the percentage increase in the intensity of the ESR signal of DMPO–OH spin-adduct and ASC•, which indicates an increase in the concentration of hydroxyl and ascorbyl compared to the control sample, are shown in Table 2.

Table 1. The levels of reducing power and glutathione peroxidase activity in the aqueous humor of patients with age-related cataracts of different maturity

Type of age-related cataract	Reducing power (mmolTE/ml)		Glutathione peroxidase (U/ml)	
	Mean age (years)			
	67.41 ± 2.89	78.23 ± 3.42	67.41 ± 2.89	78.23 ± 3.42
Cortical cataract (C group)	1.81 ± 0.07[a]	1.02 ± 0.04[a]	4.01 ± 0.14[a]	2.81 ± 0.13[a]
Nuclear cataract (N group)	0.76 ± 0.04[b]	0.66 ± 0.03[b]	2.15 ± 0.07[b]	1.72 ± 0.06[b]
Mature cataract (M group)	0.32 ± 0.08[c]	0.15 ± 0.01[c]	1.05 ± 0.04[c]	0.98 ± 0.03[c]

The values are represented as mean ± standard deviation; values sharing the same letters in the same column are not significantly different from each other at the level $p < 0.05$

Table 2. Increase in the intensity of the electron spin resonance signal of hydroxyl (HO•) and ascorbyl (ASC•-) radicals in the aqueous humor of patients with age-related cataracts of different maturity

Type of age-related cataract	Hydroxyl radical – RI HO• (%)		Ascorbyl radical – RI $ASC^{•-}$ (%)	
	Mean age (years)			
	67.41 ± 2.89	78.23 ± 3.42	67.41 ± 2.89	78.23 ± 3.42
Cortical cataract (C group)	29.45 ± 1.01[a]	36.22 ± 1.82[a]	26.12 ± 0.89[a]	36.2 ± 1.82[a]
Nuclear cataract (N group)	38.12 ± 1.29[b]	48.72 ± 1.71[b]	41.15 ± 1.39[b]	58.75 ± 2.06[b]
Mature cataract (M group)	74.14 ± 2.52[c]	85.50 ± 1.94[c]	83.56 ± 2.84[c]	90.65 ± 2.96[c]

The values are represented as mean ± standard deviation; values sharing the same letters in the same column are not significantly different from each other at the level $p < 0.05$

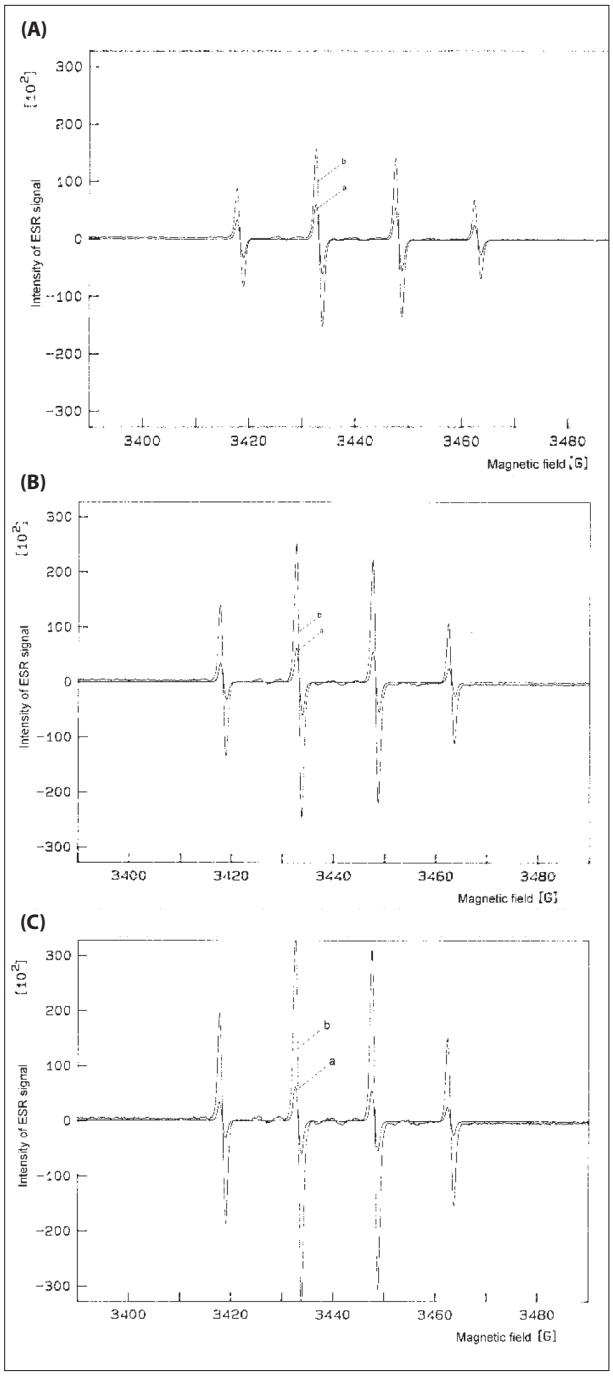

Figure 1. Electron spin resonance (ESR) spectra of 5,5 Dimethyl 1 Pyrroline 1 Oxide spin-adduct: A – a) control sample; b) aqueous humor of patient P15 with age-related cortical cataract; B – a) control sample; b) aqueous humor of patient P31 with age-related nuclear cataract; C – a) control sample; b) aqueous humor of patient P50 with age-related mature cataract

Testing the differences, depending on the cataract maturity, a significantly higher concentration of HO• was found in AH with the patients with mature cataract, and the production of HO• increases during the process of aging. The HO•concentrations in AH of patients in the 65–69-year-old age group increased for 29.45 ± 1.01% in C, 38.12. ± 1.29% in N, and 74.14 ± 2.52% in M group, respectively. In patients from ≥ 70 group, the intensity of ESR signal increase is higher (36.22 ± 1.82% in C, 48.72 ± 1.71% in N, and 85.50 ± 1.94% in M group), respectively.

In all 55 cases a typical ESR spectrum of DMPO–OH spin-adduct, with four lines of relative intensities 1:2:2:1 and hyperfine splitting constant $a_N = a_H = 14.9$ G was observed. Increase in the relative intensity of ESR signal of DMPO–OH spin-adduct expressed in percentages relative to the control sample. The examples of ESR spectra obtained in the AH of patient P15 with cortical cataract are presented at Figure 1A. Figure 1B shows the ESR signal of DMPO–OH spin-adduct detected in the AH of patient P31 diagnosed with nuclear cataract. The highest ESR signal intensity of the DMPO–OH signal was registered in patient P50 with mature cataract (Figure 1C). The mean values of the percentage increase in the ESR signal of DMPO–OH spin adducts are summarized in Figure 2.

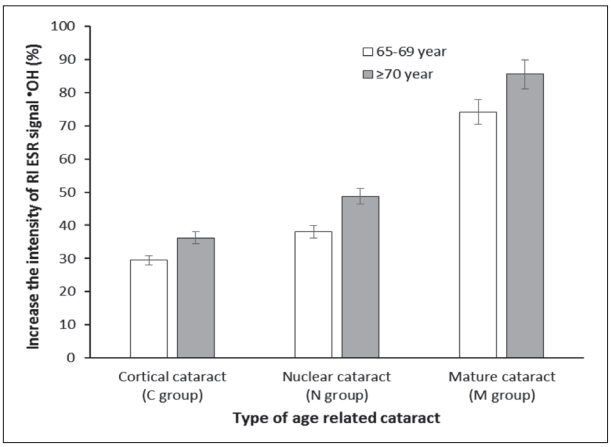

Figure 2. Mean values of the percentage increase in the intensity of the electron spin resonance signal of 5,5 Dimethyl 1 Pyrroline 1 Oxide spin-adduct during the Fenton reaction in the presence of aqueous humor of patients with age-related cataracts of different maturity compared to the control sample

The ASC• concentrations in AH in the 65–69-year age group increased by 26.12 ± 0.89% in C, 41.15 ± 1.39% in N, and 83.56 ± 2.84% in M group, respectively. In the group of patients over 70 years old, the increase in the intensity of the ESR signal of ASC• is much higher (36.20 ± 1.82% in C, 58.75 ± 2.06% in N, and 90.65 ± 2.96% in M group), respectively (Table 2).

The free radicals obtained directly in AH of patients with cataract were characterized by ESR spectroscopy as a simple doublet showing coupling constants of AH = 1.84 G. According to literature data this can be assigned to an ASC• [12]. The unpaired electron in the structure of the ASC• is located in the π-system that includes the tri-carbonyl group of ascorbates. Thermodynamically, it is relatively unreactive with a one-electron reduction potential of only +282 mV. The examples of typical ESR spectra obtained in the AH of patients with cortical, nuclear, and mature cataract, are presented at Figure 3A, 3B, and 3C. The mean values of the percentage increase in the ESR signal of ASC• are summarized in Figure 4.

The concentrations of HO• were significantly positively correlated to the concentrations of ASC• ($r = 0.8355$; $p < 0.05$) (Figure 5) in AH of patients with age-related

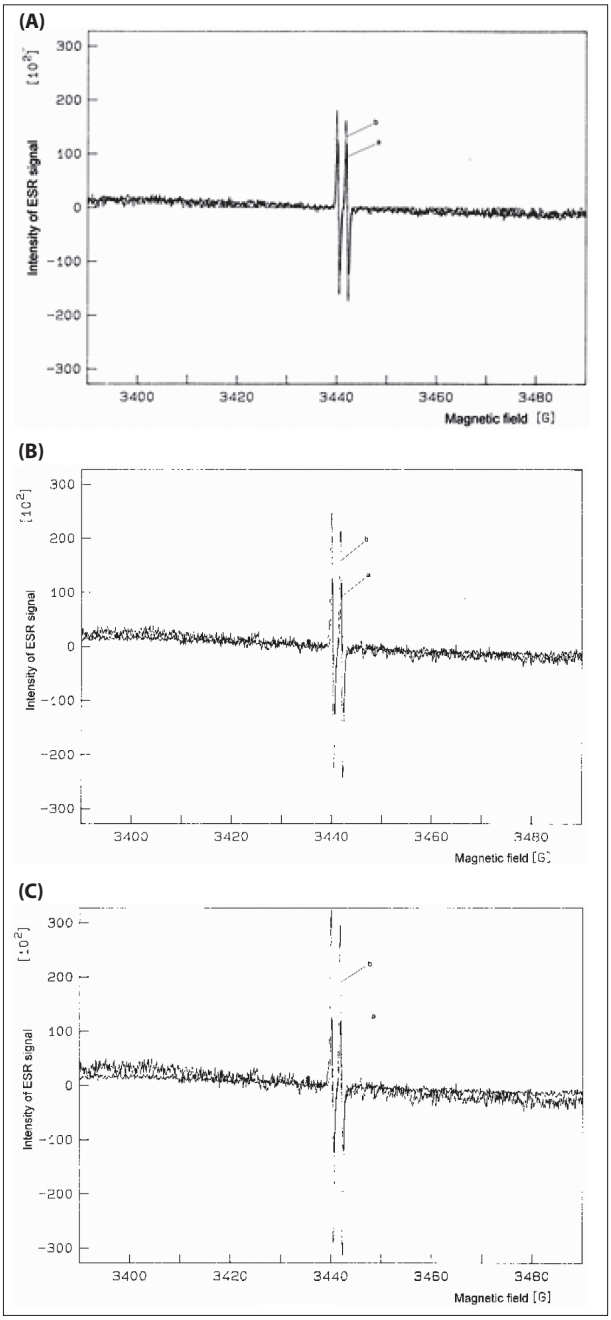

Figure 3. Electron spin resonance (ESR) spectra of ascorbyl radical: A – a) control sample; b) aqueous humor of patient P15 with age-related cortical cataract; B – a) control sample; b) aqueous humor of patient P31 with age-related nuclear cataract; C – a) control sample; b) aqueous humor of patient P50 with age-related mature cataract

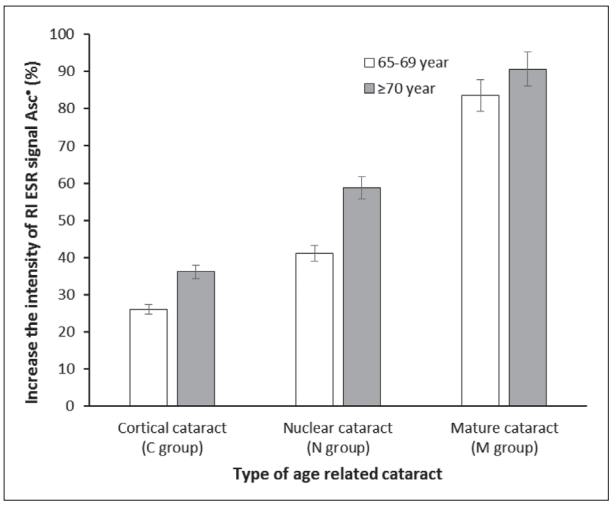

Figure 4. Mean values of the percentage increase in the intensity of the electron spin resonance (ESR) signal of ascorbyl radical obtained in the presence of aqueous humor of patients with age-related cataracts of different maturity compared to the control sample

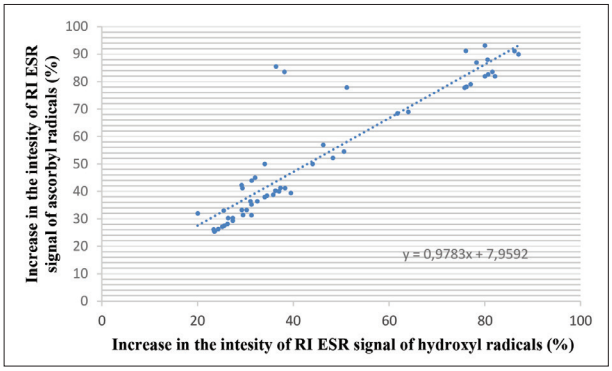

Figure 5. Correlation between the concentrations of ascorbyl radical and the concentrations of hydroxyl radical in aqueous humor of patient with age-related cataracts of different maturity

cataract while the activity of GPx significantly negatively correlated with the increase in the concentration of HO• ($r = -0.7590$; $p < 0.05$).

DISSCUSION

This study showed, that the level of oxidants and antioxidants present in AH, in addition to the age of the patients, is also significantly influenced by the maturity of the cataract. We emphasize on the GPx activity changes seen in the AH of individuals with incipient cataract (cortical group C, 18 patients; nuclear group N, 20 patients, and mature cataract group M, 17 patients). Our results indicate a significant reduction of GPx concentrations in patients with nuclear cataract compared to the patients with cortical cataract (Table 1). Chronic lens exposure to molecular oxygen conditions the decline in the status of antioxidant enzymes, which conditions the pathogenesis of nuclear cataract [15]. This fact is responsible for increased OxS that causes protein damage in the lens core, protein aggregation, light scattering and loss of lens transparency. Compounds with reducing ability, as electron donors, break the chain of radical reactions by converting free radicals into non-radical products. Since it is in close contact with the cornea, anterior chamber, trabecular meshwork, and lens, the RP capacity in the AH has an impact on their health [16].

Changes in GPx activity could significantly impact the steady state concentration of H_2O_2. H_2O_2 is typically present in the AH and is harmful to cells. Although H_2O_2 is a vital part of many signal-transduction pathways, the antioxidant enzymes catalase and GPx eliminate it when its levels rise over healthy limits. Due of its capacity to decrease both inorganic and organic peroxides, GPx may have a higher level of oxidant homeostasis than catalase.

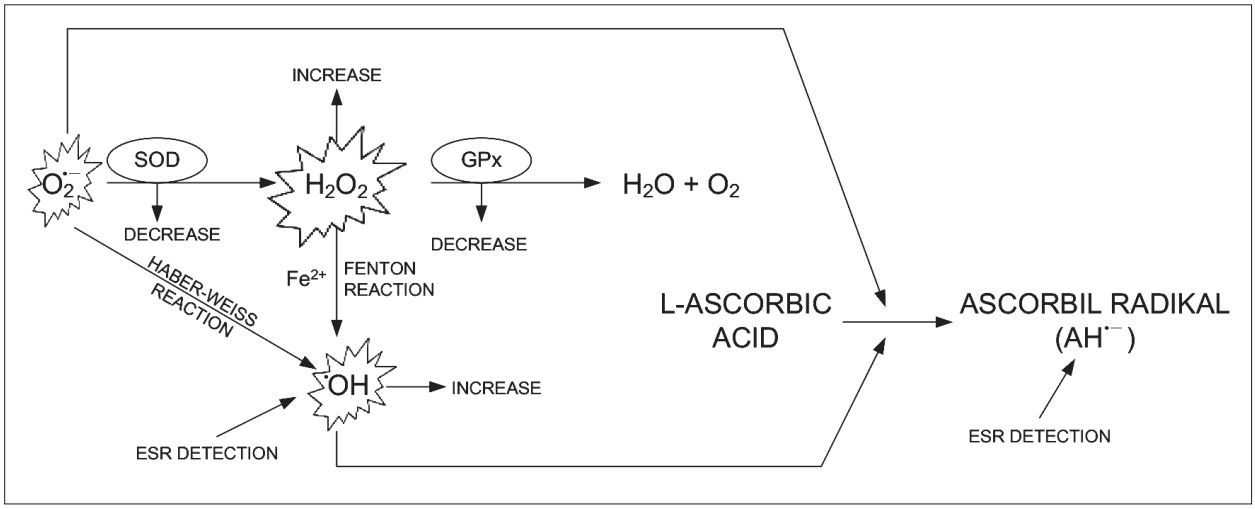

Figure 6. Scheme of free radical/antioxidant imbalance in the aqueous humor of patients with age-related cataract

This could result in glutathione depletion because GPx needs glutathione as a cofactor to remove H_2O_2. Ascorbic acid metabolism depends on glutathione, and its depletion results in ASC• that cannot be converted back into ascorbic acid [17, 18]. Our findings support this theory, since OxS brought on by elevated levels of free radicals decreases GPx activity and RP (Table 1).

With the progression of age-related cataracts, from incipient to mature, the capacity of the lens to stimulate HO• production increases (Table 2) and dramatically change antioxidant status of the AH.

Free translation of metal ions and iron complexes in hemoglobin, myoglobin, lactoferrin, and transferrin may catalyze a variety of pathogenic processes [19]. The concentration of iron and copper ions is lower in lenses without cataracts, while the increase in their concentration affects the increase in the production of HO•. *In vitro* systems have been used extensively to study their capacity for creating HO• through the Fenton reaction ($H_2O_2 + M^{(n-1)+} \rightarrow HO^{\bullet} + HO^- + Mn^{n+}$). This suggests that HO• damage plays a role in the development of age-related cataract [20]. Also, oxidation of lipoprotein increase [21], while copper-zinc superoxide dismutase activities decreases due to the accelerated generation of ROS, especially HO•. When enzyme activities are lost or diminished, H_2O_2 and free radicals can cause the lens to irreversibly degrade, including a decrease in Na-K ATPase activity [22].

In this work, we examined the direct generation of HO• in AH at different stages of cataract severity. Our results direct evidence that the largest concentration of HO• detected. In all patients with age-related cataract (C, N, and M group) Increase in the concentration of HO• in AH causes the process of oxidation (Figure 2).

During aging, the content of ascorbic acid decline and their transport into the ocular humor is difficult. ASC•, which have minimal reactivity, are created when ascorbic acid reacts with oxygen radicals. In a nutshell, an increase in OxS is correlated with an increase in ASC• concentration. In brief, an increase in ASC• concentration correlates with an increase in OxS.

Several factors need to be considered in order to understand the biochemical mechanisms that may underlie this observation. The reaction of ascorbate monoanion ($AscH^-$) with superoxide anion radical leads to the formation of ASC• ($AscH^{\bullet-}$). But the reactivity of the superoxide anion is insufficient to explain the damage observed in biological systems. However, many of the harmful effects of superoxide anion are indirect and result from its chemical transformation into a HO•. Because of that we investigated the presence of HO• in AH of patients with age-related cataract incipient (groups C and N) and mature (Table 2, Figure 4). HO• is a potent oxidizing agent with very high-rate constants (10^9–10^{10} M^{-1} s^{-1}) for H-abstraction on this reaction $HO^{\bullet} + AscH^- \rightarrow Asc^{-\bullet} + H_2O$. Additionally, as a cataract develops, the loss of ascorbic acid pathways through ROS and oxidized metals (Cu^{2+} or Fe^{3+}) reactions are potentially conceivable [23]. All proposed mechanisms produce ASC• which form dehydroascorbic acid as an end product of oxidation. Based on this, a hypothetical model of free radicals in the AH of patients with age-related cataract based on the results of the current study was constructed (Figure 6).

Lipid peroxidation has been proposed as a causative factor of cataract, which will be the subject of our next investigations and also further study is needed to establish some other free radicals which is included into the pathogenesis of age-related cataract. Although this study is limited by small sample size, the results show that the majority of AH OxS markers can be connected to the maturity stage of age-related cataract.

CONCLUSION

The AH protects the inner parts of the eye against the damaging effect of reactive oxygen species generated by them. This is possible due to the effective antioxidant protective mechanisms. According to our results, the RP and GPx activity concentrations were significantly reduced, while the intensities of ESR signals of HO• and ASC• increase

during the aging process and depend on the maturity of the cataract. AH OxS markers and antioxidants are believed to mirror the intrinsic oxidant/antioxidant balance of the surrounding eye tissues. The presented results suggest that the maturity of cataract should be taken into account in biochemical studies of ocular OxS.

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Note: The results were presented at The First Forum of the Academy of Medical Sciences of the Serbian Medical Society and published as an abstract in the announcement from that meeting in Srp Arch Celok Lek. 2023 Jan–Feb;151(1–2):120–34.

Conflict of interest: None declared.

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Антиоксиданти и слободни радикали у очној водици болесника са сенилном катарактом

Стефан Брунет[1,2], Никола Бабић[1,2], Софија Давидовић[1,2], Александар Миљковић[1,2], Никола Кнези[2], Владимир Чанадановић[1,2,3]

[1]Универзитетски клинички центар Војводине, Очна клиника, Нови Сад, Србија;
[2]Универзитет у Новом Саду, Медицински факултет, Нови Сад, Србија;
[3]Академија медицинских наука Српског лекарског друштва, Београд, Србија

САЖЕТАК

Увод/Циљ Сенилна катаракта је један од најчешћих узрока оштећења вида широм света. Оксидативна оштећења и слободни радикали значајно утичу на етиопатогенезу катаракте. Циљ овог истраживања је био одређивање антиоксидативног капацитета и оксидативног стреса у очној водици пацијената у односу на старосну доб и зрелост катаракте.

Методе Клиничка и биохемијска испитивања су спроведена код 55 пацијената са сенилном катарактом. Према степену зрелости катаракте, сви пацијенти су подељени у инципиентне групе (кортикална – група *C*, 18 болесника; нуклеарна – група *N*, 20 болесника и зрела – група *M*, 17 болесника). У односу на старосну доб формиране су две групе: Група I (65–69 година) и Група II (70 ≥ година). Антиоксидативна активност у очној водици је мерена методом редукционе способности и активности ензима глутатион-пероксидазе (*GPx*). Промене концентрација хидроксилних и аскорбилних радикала детектоване су електронском спиналном резонантном спектроскопијом.

Резултати Редукциона способност и *GPx* активност су статистички значајно смањене ($p < 0,001$) у групи *N* у поређењу са групом *C* и у групи *M* у поређењу са групом *N*. Концентрације хидроксилних (29,45 ± 1,01% у *C*, 38,12 ± 1,29% у *N* и 74,14 ± 2,52% у групи *M*) и аскорбилних радикала (26,12 ± 0,89% у *C*, 41,15 ± 1,39% у *N* и 83,56 ± 2,84% у *M*) значајно су порасле ($p < 0,05$) са прогресијом катаракте и старосном доби болесника. Утврђена је значајна негативна корелација ($r = -0,759$, $p < 0,05$) између концентрације хидроксилних радикала и садржаја *GPx*.

Закључак Наше истраживање је доказало да на ниво оксидативног стреса у очној водици поред старости значајно утиче и степен зрелости катаракте. Добијени подаци подржавају хипотезу да се током старења, у зависности од зрелости катаракте у очној водици, антиоксидативни капацитет смањује услед повећања концентрације реактивних хидроксилних радикала.

Кључне речи: катаракта; антиоксидативни ензим; хидроксилни радикал; аскорбилни радикал

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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Depression and distress in couples with infertility – Who suffers more?

Bojana Pejušković[1,2], Oliver Tošković[3], Milica Ivanišević[2], Marija Lero[1], Otaš Durutović[2,4]

[1]Institute of Mental Health, Clinical Department for Crisis Interventions and Affective Disorders, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[3]University of Belgrade, Faculty of Philosophy, Belgrade, Serbia;
[4]University Clinical Centre of Serbia, Institute of Urology and Nephrology, Belgrade, Serbia

SUMMARY

Introduction/Objective Infertility is the inability to achieve pregnancy after a year or more of unprotected sexual intercourse. It is a clinical and social issue affecting both sexes. Infertility can cause anxiety, depression, and personal distress with long-lasting consequences. Men and women tend to cope with infertility in different ways and reliance on certain coping mechanisms can be harmful.
This study aims to examine the correlative effects of infertility, distress and depression among couples, and investigate sex disparities in levels of suffering.

Methods The research is a cross-sectional study that included 168 participants (84 couples) divided into two groups, control and infertility group. Beck Depression Inventory Second Edition (BDI-II) and the Brief Symptom Inventory (BSI) were used to identify and assess psychological symptoms. Statistical analysis was performed using SPSS at the 0.05 level of significance.

Results The results showed that there was a significant difference in the scores on BDI and BSI scales between the infertile and fertile groups, with participants in the infertile group reporting higher levels of depression and distress ($t = -2.724$, $df = 166$, $p < 0.01$; $t = -3.609$, $df = 166$, $p < 0.01$). Women had significantly higher scores on the depression scale than men ($t = -2.079$, $df = 166$, $p < 0.05$).

Conclusion In summary, the study found that couples dealing with infertility experience higher levels of distress and depression compared to the control group. Women in these couples are particularly vulnerable to depression. The study highlights the importance of addressing the mental health needs of individuals and couples dealing with infertility, in addition to treating the underlying medical issues.

Keywords: infertility; depression; anxiety; male; female

INTRODUCTION

In the 11th revision of the International Classification of Diseases (ICD-11) infertility is defined as a disease of the male or female reproductive system defined by the inability to achieve pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. So far it remains a comprehensive health issue which affects millions of people of childbearing age. Despite efforts to address this worldwide problem, infertility continues to rise in many countries, regardless of the socio-demographic index [2]. It remains a significant personal, clinical, and social issue that affects both men and women globally.

Infertility is a complex issue with numerous factors contributing to its development. These factors can be classified into four standard categories: female factors, male factors, combined causes, and unexplained infertility. Psychological factors and fertility difficulties have reciprocal cause-effect relationships, potentially leading to devastating effects on one's mental wellbeing. In addition to being classified as a disease, infertility is often viewed as an inability to fulfill a biological and social role of producing offspring, particularly in traditional societies where it is seen as a significant responsibility for both men and women. These social expectations can be distressing and create restlessness, which can lead to various mental health issues [3]. Furthermore, infertility as a health issue, coupled with the various forms of therapy that infertile couples frequently undergo, often resulting in a series of failures, can cause couples to constantly fluctuate between confidence and hopelessness, which creates conditions for stress to develop. Higher levels of personal distress and lower quality of life that can emerge as consequence of such events represent a risk factor for post-traumatic stress disorder [4]. Approaching infertility and its treatment in an emotionally unstable state, whether it originates from before infertility awareness or from the infertility itself, can increase the likelihood of giving up, feeling defeated, and being unwilling to continue pursuing the goal of conceiving, ultimately leading to a lack of endurance in the infertility management process [5]. These findings emphasize the crucial role of mental health in individuals struggling with infertility.

Studies have demonstrated that men and women tend to cope with infertility as a life-

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Correspondence to:
Bojana PEJUŠKOVIĆ
Institute of Mental Health
Clinical Department for Crisis Interventions and Affective Disorders
Milana Kašanina 3
11000 Belgrade, Serbia
bpejuskovic@hotmail.com

crisis moment in different ways [6, 7]. While coping mechanisms are essential in managing stress they can be inadequate and even exacerbate it [8]. One study found that women tend to rely on escape/avoidance strategies, while men tend to prefer distancing and planful problem-solving. The study also revealed a correlation between the use of escape/avoidance as a coping mechanism and higher levels of reported stress in individuals [9]. This suggests that relying on such strategies may be potentially harmful and unhelpful in overcoming infertility-related psychological difficulties. These findings and correlations are beneficial in understanding fundamental differences between men and women in terms of approaching infertility as a disease, perceiving it as an incompetence, and acknowledging potential discrepancies in stress levels and emotional despair between sexes.

Distress and emotional instability can create a vicious circle with infertility, leading to changes in reproductive function [10]. Being unable to fulfill the biological and social role of providing a progeny despite a desire to do so can result in frustration, a life crisis, emotional disequilibrium and social stigma [11]. Although the social component is typically emphasized in conservative and developing countries, the impact of infertility on mental health is observed in developed countries as well [12]. Infertility is a multidimensional stressor that causes anxiety, depression, and personal distress, and its consequences can be long-lasting, even after achieving parenthood [13].

As mentioned earlier, psychological factors and fertility difficulties have a reciprocal cause-effect relationship, with significant effects on mental health. Therefore, the aim of this study was to examine the correlative effects of infertility, distress, and depression among couples with infertility. Moreover, given the differences in the way sexes cope with this disability and overcome stress, we aim to investigate whether there is a disparity in levels of suffering between men and women.

METHODS

Participants

In order to conduct this study, we formed the sample that included 168 participants (84 couples). The participants were divided into two groups: (a) infertile group (index group, N = 84) and (b) fertile group (control group, N = 84). The infertile group comprised 42 couples, who were patients at the Clinic of Urology, University Clinical Centre of Serbia. All couples have been trying to achieve pregnancy for at least 12 months before starting their infertility related examinations. Male patients, married or in an extramarital union, came with their female partners to examine the cause of infertility. Female reasons of infertility were not yet excluded. The examination involved semen analysis, which was free at the time. All infertile couples that have undergone a test during 2013. were included, regardless of their fertility testing results. When it comes to the fertile group, it consisted of couples with children. They were selected randomly in the kindergarten institution. Couples from infertile and control groups were not matched according to socio-demographic characteristics.

Procedures and measures

The research was designed as a cross-sectional study. Before joining the research, all respondents were acquainted in detail with the course and significance of the research, as well as their role, obligations, and rights. In accordance with the above, the participants have signed an informed consent to participate in the research before starting the research process.

Data on the participants' age, sex, and marital status were obtained through a brief structured socio-demographic questionnaire.

To identify and assess depressive symptoms, we used The Beck Depression Inventory Second Edition (BDI-II) [14]. It contains a 21-item self-report instrument that intends to assess the presence and severity of symptoms of depression as listed in the American Psychiatric Association`s Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). Each of the 21 items, that correspond to a symptom of depression, is summed to give a single score for the BDI-II. There is a four-point Likert-type scale for each item, ranging from 0 to 3. On two items (16th and 18th) there are seven options to indicate an increase or decrease of appetite and sleep. Cut score guidelines for the BDI-II are given with the recommendation that thresholds can be adjusted based on the characteristics of the sample and the purpose of the use of the BDI-II. Total score of 0–13 is considered minimal range, 14–19 mild, 20–28 moderate, and 29–63 is severe.

To identify self-reported clinically relevant psychological symptoms among participants, we used The Brief Symptom Inventory (BSI) [15]. It consists of 53 items covering nine symptom dimensions: Somatization, Obsession-Compulsion, Interpersonal Sensitivity, Depression, Anxiety, Hostility, Phobic anxiety, Paranoid ideation, and Psychoticism; and three global indices of distress: Global Severity Index, Positive Symptom Distress Index and Positive Symptom Total. The global indices measure current or past level of symptomatology, intensity of symptoms, and number of reported symptoms, respectively. Respondents rank each feeling item (e.g., "your feelings being easily hurt") on a five-point scale ranging from zero (not at all) to four (extremely). Rankings characterize the intensity of distress during the past seven days.

The research was conducted according the principles of good scientific practice with the approval of the Ethics Committee of the University Clinical Centre of Serbia (No. 1040/29) while all obtained data are kept confidential.

Statistical analysis

The obtained data was imputed into IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY, USA). Descriptive statistics indicators, that is, measures of central tendency [arithmetic mean ($\bar{x}$)] and measures

of variability [standard deviation (SD)] were used to analyze socio-demographic characteristics of participants. For further data processing we conducted analysis of variance, multiple regression, T test and correlations. Analysis was done on the level of significance (α level) 0.05.

RESULTS

The findings of the data collected from the participants can be summarized under two sections: results showing differences between the two groups – infertile and fertile (A) and results showing the sex differences (B).

Our sample included 168 participants (84 couples), adults, aged 20–46 years (($\bar{x}$ = 35.23, sd = 4.974).

Results showing differences between infertile and fertile groups indicate a significant difference in the scores on BSI and BDI scales ($p < 0.05$). T test for independent samples, which tested difference between these groups, shows significant relation of BSI and BDI scores with infertile group ($t = -2.724$, $df = 166$, $p < 0.01$; $t = -3.609$, $df = 166$, $p < 0.01$). These results show that participants form the infertile group have higher levels of depression and distress compared to the control group (Figure 1, Table 1).

Results showing the sex differences indicate that women have statistically significantly higher scores on the depression scale compared to men. T test for independent samples which tested difference between sexes shows higher BDI scores among females ($t = -2.079$, $df = 166$, $p < 0.05$). Our results show that females cope with depression of higher intensity, compared to the male population of our sample (Figure 2).

DISCUSSION

A cross-sectional study was conducted to investigate the prevalence of mental health issues, specifically depression and distress, among patients facing infertility. Additionally, the study aimed to explore potential sex differences in depression and distress rates. Given the growing increase in infertility, this research provided critical insights to better understand the risks and consequences associated with this condition. The results of the study revealed significantly higher levels of both depression and distress among participants in the infertile group compared to the group of fertile couples. Moreover, the results indicated higher scores on the depression scale among women in the infertile group.

Our study observed higher rates of depression and distress in individuals experiencing infertility. Such findings are well-documented and can be attributed to the significant stress associated with the inability to conceive children [16]. However, it is important to acknowledge that the psychological burden experienced by infertile individuals extends beyond just the physical aspects of their condition. Some studies showed that social stigma surrounding infertility can further exacerbate mental health issues [11] resulting in a deepening of psychological distress associated with the disease.

Table 1. Descriptive statistics results for Brief Symptom Inventory (BSI) and Beck Depression Inventory (BDI) scales results

Scale	Group	N	Mean	Std. deviation	Std. error mean
BSI_total	control	84	21.7262	20.94994	2.28583
	infertility	84	32.7738	30.71178	3.35093
BDI_total	control	84	4.1548	3.88849	0.42427
	infertility	84	7.2381	6.79762	0.74168

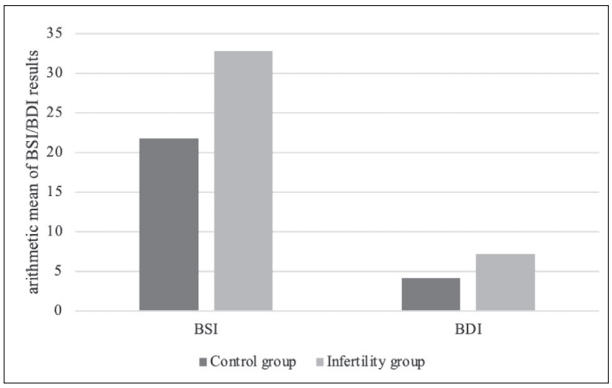

Figure 1. Graphic display of Brief Symptom Inventory (BSI) and Beck Depression Inventory (BDI) scores in the control and the infertility group

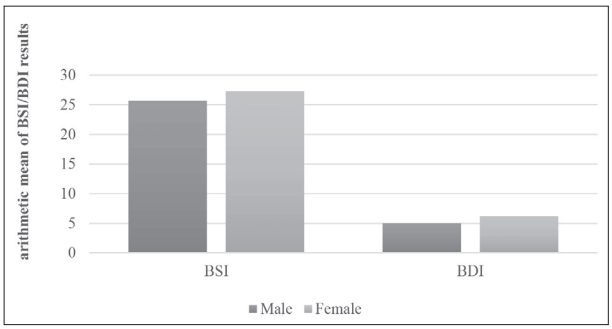

Figure 2. Graphic display of Brief Symptom Inventory (BSI) and Beck Depression Inventory (BDI) scores among males and females in the infertility group

Depression is one of the most common mental health issues worldwide and significantly impacts an individual's everyday functioning and quality of life. Among individuals experiencing infertility, studies have reported higher depression levels, even comparable to those of patients diagnosed with cancer, highlighting the severity of the psychiatric consequences associated with infertility [17]. This emphasizes the importance of prioritizing mental health when providing healthcare to individuals and couples experiencing infertility. Our findings are consistent with numerous previous studies, which have demonstrated that the inability to achieve parenthood leads to depressive symptoms [18, 19].

These findings are understandable, given the lower self-esteem, sexual performance, and confidence reported by infertile men compared to control groups, as well as negative effects on women's self-esteem and sexual activity [20, 21]. In fact, a study examining communication among women with infertility revealed that many women discuss infertility only with close family and friends, and that 7%

of these women were unable to discuss the issue with their spouse [22]. Moreover, infertility-related stigma plays an important role in marital distress in infertile women which contributes to marital communication difficulties leading to worsening of mental health issues [23].

Most studies examining the link between mental health and infertility have focused primarily on women's health. For example, a prospective study involving 416 women with infertility revealed that the more depressed an infertile woman is, the less likely she is to begin infertility treatment, and the more likely she is to drop out after only one cycle [5]. These findings underscore the extent to which infertility and mental health are inextricably intertwined. Researchers have also demonstrated that discontinuation of treatment is most often due to psychological reasons, despite a good prognosis and adequate financial resources to pay for treatment [24]. Moreover, numerous studies have highlighted the impact of mental health on treatment outcomes, with lower pregnancy rates observed in women who are more distressed prior to and during treatment [25, 26].

Our study showed that females are more prone to depressive symptoms compared to men in infertile couples. Palomba et al. [27] examined the influence of stress and quality of life on female fertility and found that distress-mediated symptoms of infertility affect more women than man, which stands in agreement with our findings. In their study, findings were related to lifestyle choices, more precisely due to focusing on career, education, and waiting for the right moment of motherhood [27].

Recent findings also indicate that, even though this disability affects both sexes, women are more strongly affected by this disability compared to men due to higher social stigma [28, 29]. A recent review conducted by Xie et al. [30], that included numerous studies regarding social stigma in infertility, emphasized that women are more prone to distress and psychological burden of infertility stigma compared to men. Moreover, a sex difference for this variable can as well be explained by the fact that men often hide psychological problems and are reluctant to report symptoms. Namely, masculine prototype incited anger, which led to hiding all the feelings that can be declared feminized. Moreover, in many societies, regardless of infertility being caused by male factors, women are the ones that are blamed and marked in society, which intensifies mental struggle and leads to worsening of distress and depressive symptoms among them.

The study has limitations concerning the moderately small sample size and single-center experience. In addition, sociodemographic matching has not been made between participants in examined groups.

CONCLUSION

The results of this study demonstrate that couples with infertility experience significantly higher levels of distress and depression compared to the control group. Moreover, women in these couples are particularly vulnerable to depression. These findings emphasize the importance of addressing the mental health needs of individuals and couples dealing with infertility, in addition to treating the underlying medical issues. Without appropriate psychological care and counseling, individuals may be at risk of serious social and emotional consequences, and may struggle to cope with the challenges of infertility treatment. As such, a multi-disciplinary approach is necessary to help couples achieve their goal of parenthood while also addressing their mental health needs. By providing psychological support and care, healthcare providers can help to prevent long-lasting psychological disruptions and promote better overall functioning and quality of life for individuals dealing with infertility. Ultimately, addressing mental health concerns can have a positive impact on the fertilization process and increase the likelihood of successful treatment outcomes.

Conflict of interest: None declared.

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Депресија и стрес код парова са инфертилитетом – ко пати више?

Бојана Пејушковић[1,2], Оливер Тошковић[3], Милица Иванишевић[2], Марија Леро[1], Оташ Дурутовић[2,4]

[1]Институт за ментално здравље, Клиничко одељење за кризна стања и афективне поремећаје, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија;
[3]Универзитет у Београду, Филозофски факултет, Београд, Србија;
[4]Универзитетски клинички центар Србије, Клиника за урологију, Београд, Србија

САЖЕТАК

Увод/Циљ Инфертилитет представља немогућност остваривања трудноће после годину дана незаштићених сексуалних односа. Овај клинички и социолошки проблем погађа оба пола. Неплодност може довести до анксиозности, депресије и дистреса са дугорочним последицама. Мушкарци и жене се често другачије суочавају са овим проблемом, а ослањање на извесне механизме одбране може бити штетно. Циљ ове студије био је испитивање међузависних односа неплодности, депресије и дистреса, као и испитивање разлике међу половима у нивоу патње узроковане неплодношћу.

Методе Спроведена је студија пресека која је укључивала 164 учесника (84 пара) подељена у две групе – контролну и групу са инфертилитетом. За детекцију и процену психолошких симптома коришћени су друга верзија Бековог инвентара депресивности (енг. *Beck Depression Inventory* (*BDI*-II)) и Кратки инвентар симптома (енг. *The Brief Symptom Inventory* (*BSI*)). Статистичка анализа вршена је у статистичком софтверу *SPSS* на нивоу значајности 0,05.

Резултати Резултати су показали да постоји статистички значајна разлика у скоровима на *BDI* и *BSI* скали између две испитиване групе, где неплодни парови пријављују више нивое депресије и дистреса (t = -2,724, df = 166, p < 0,01; t = -3,609, df = 166, p < 0,01). Међу неплодним паровима, жене су имале више нивое депресије од мушкараца (t = -2,079, df = 166, p < 0,05).

Закључак Студија је показала да инфертилни парови имају значајно више нивое депресије и дистреса од парова из контролне групе. Нарочито су подложне депресији жене из групе парова са инфертилитетом. Студија показује значај процене и одржавања менталног здравља код неплодних парова у циљу лечења самог инфертилитета.

Кључне речи: инфертилитет; депресија; стрес; мушки пол; женски пол

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PRELIMINARY COMMUNICATION / ПРЕТХОДНО САОПШТЕЊЕ

The advantage of endoscopic treatment of Haglund's syndrome with the three-portal technique

Miodrag Glišić[1,2], Vladan Stevanović[1,2], Aleksandar Jevtić[1,2], Milan Mirković[1], Ljubomir Daković[1], Angelica Arce Stanković[1], Sanja Mirković[3], Lazar Mičeta[1,2]

[1]Banjica Institute for Orthopedics, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[3]University of Belgrade, Faculty of Sport and Physical Education, Belgrade, Serbia

SUMMARY

Introduction/Objective Heel deformity accompanied by pain at the attachment of the Achilles tendon is generally known as Haglund's syndrome. The prominence of the posterosuperior part of the heel bone generates pressure on the retrocalcaneal bursa and Achilles tendon, causing swelling and pain. The condition itself can be treated using surgical or non-surgical methods. The aim was to present our first experiences in the endoscopic treatment of Haglund's syndrome, employing the three-portal technique.

Methods This study includes ten patients whose surgeries were performed during the period between January 2019, and May 2020. All interventions were endoscopic with the three-portal technique used. The diagnosis was made based on the anamnesis, clinical examination, X-rays, and magnetic resonance imaging diagnostics. For the evaluation of results, the AOFAS (American Orthopedic Foot and Ankle Society) score was used.

Results The endoscopic findings in all patients revealed a hypertrophic retrocalcaneal bursa and prominence at the posterosuperior part of the calcaneus, generating pressure on the Achilles tendon. By employing the three-portal technique, considerably better visualization is obtained, enabling easy removal of the degenerated tissue. The radiographic control image was satisfactory. The result of the AOFAS score showed a significant improvement after the surgery.

Conclusion The endoscopic approach and the use of the three-portal technique in resolving Haglund's syndrome is a secure procedure that produces good results. It enables faster recovery and fewer complications compared to open surgery.

Keywords: endoscopic calcaneoplasty; calcaneus; Haglund's syndrome; retrocalcaneal bursitis

INTRODUCTION

Haglund's syndrome is the condition accompanied by a pain at the backside of the heel, and by a deformity of the heel bone at the place of Achilles tendon. The condition was first described by Haglund in 1928 [1]. The "pump-bump" deformity of the calcaneus, occurring as a prominence of the posterosuperior part in front of the Achilles tendon attachment, generates the pressure on the surrounding retrocalcaneal bursa and Achilles tendon. Over time, this results in the retrocalcaneal bursa growth and the tendon damage. The pain most often occurs at the beginning of movement after a period of rest, and is related to the use of tight footwear too. The pain is caused by the pressure around the Achilles tendon attachment, as well as the dorsiflexion of the foot. There are numerous theories to explain the etiology of this condition: use of tight footwear, overload while running, hereditary factors, and disturbed biomechanics of the subtalar joint [2]. Various types of non-surgical and surgical treatment are applied for this condition. Non-operative treatment includes: wearing wide shoes, heel lifts, stretching exercises, physical procedures, and the local application of corticosteroid injections [3]. If conservative treatment does not provide good results after six months, a surgical approach is considered [4]. The goal of the surgical treatment is to remove the heel bone lump that causes the pressure, as well as the altered retrocalcaneal bursa. The two common methods of surgical treatment today – open surgery and endoscopy. Corrective osteotomy of the heel bone is described in the literature, aiming at changing the angle of the Achilles tendon attachment, thereby releasing the pressure on the tendon [5]. Open surgery is widespread and there are numerous papers with good postoperative results [6, 7]. However, the endoscopic treatment has its advantages as it provides faster recovery with lower rate of surgical wounds complications, and it is increasingly the method of choice [8, 9].

This paper aims to present our first experiences in the endoscopic treatment of Haglund's syndrome utilizing the three-portal technique.

METHODS

In the period between January 2019 and May 2020, 10 endoscopic surgeries of remodeling the heel bone with the retrocalcaneal bursa resection were performed. The study involved

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Correspondence to:
Lazar MIČETA
Institute for Orthopedics Banjica
Mihaila Avramovića 28
11040 Belgrade
Serbia
lmiceta@yahoo.com

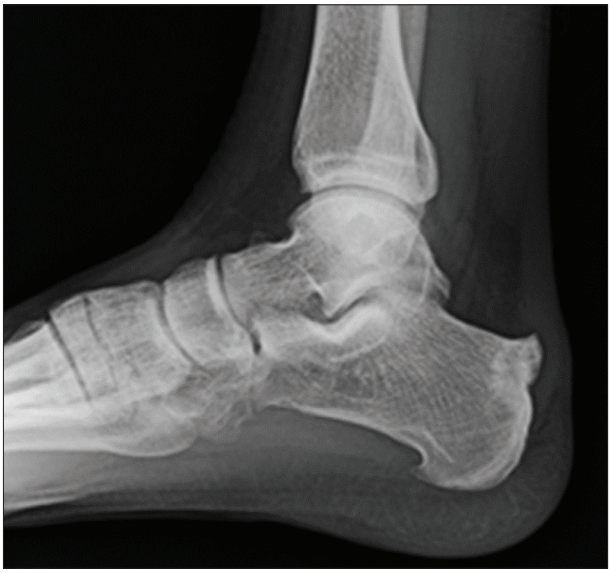

Figure 1. X-ray of Haglund's bone deformity

six men and four women of an average age of 37 years (25–66 years). All patients had suffered from the condition for more than 12 months (12–26 months) and had been treated by non-surgical methods before.

The diagnosis was confirmed by anamnestic data, clinical examination, X-ray, and magnetic resonance imaging. The anamnesis stated the presence of pain at the back of the heel, related to tight footwear and prolonged walking, especially uphill. There was a lump in the region of Achilles tendon attachment, accompanied by pain on pressure in front of the tendon's insertion point. The foot dorsiflexion was very painful.

In all patients, significant prominence at the posterosuperior part of the heel bone was presented on a lateral X-ray, although the parallel pitch lines sign was not always positive (Figure 1) [10]. Magnetic resonance imaging was taken in several patients and showed the heel bone deformity and a hypertrophic retrocalcaneal bursa with signs of initial degenerative changes of the Achilles tendon.

The study did not include patients with the Achilles tendon calcification, its partial, or full rupture, pes cavus and valgus foot deformity, or rheumatic diseases, as well as patients who had previous surgery in this region.

The AOFAS score was used to evaluate the functional results. The patients were tested before and six months after surgery [11]. The difference was statistically analyzed by Wilcoxon signed-rank test using. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 28.0 (IBM Corp., Armonk, NY, USA).

Surgical technique

All the surgeries were performed under general anesthesia, with the patient in a prone position. Initially, an X-ray was performed to check the position and degree of resection, later this was done only at the end of the operation. The instruments used included the 4 mm arthroscope with a 30° angle and a set of knee arthroscopy instruments. The three-portal technique was used: the posterolateral distal portal (DPLP), the posteromedial distal portal (DPMP), and the posterolateral proximal portal (PPLP) (Figure 2a) [12]. The PPLP was used for visualization, and the two others were working portals. After opening the portals, the first tissue in sight is a hypertrophic retrocalcaneal bursa, which is being removed by a shaver after (Figure 2b). Further on, there is a clear insight of the heel bone with a prominence at the posterosuperior part, as well as the Achilles tendon with its attachment (Figure 2c). Using the bone abrader, a prominent part of the calcaneus, which makes the pressure on the Achilles tendon, is resected (Figure 2d). The degree of the bone resection is controlled by a direct visualization of the Achilles tendon contact area to the calcaneus, with the foot in dorsiflexion. Experience in endoscopic surgery is necessary.

A lateral X-ray is performed at the end of the surgical procedure, to verify the final resection (Figure 3).

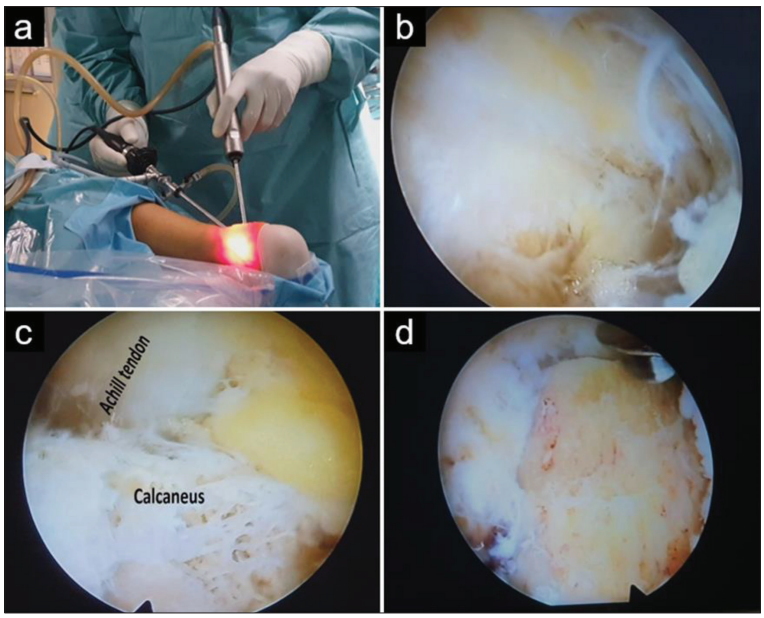

Figure 2. a – Posterolateral proximal portal and posterolateral distal portal used during the endoscopy; b – retrocalcaneal bursa; c – Achilles tendon and Haglund's deformity; d – heel bone at the place of resected deformity

Post-operative rehabilitation

Initial rehabilitation of these patients includes ankle movements, by the use of continuous passive motion machine, and partial weight-bearing starting on the first day after the surgery. Full weight-bearing was allowed in the third week after surgery. Physical rehabilitation is performed to restore the range of ankle motion and to strengthen the calf muscles. Wide shoes and absence of physical activities are suggested for three months.

The study was performed according Declaration of Helsinki ethical principles. This study was approved by committee on ethics of the Banjica Institute for Orthopedics (Resolution no. i-113/12).

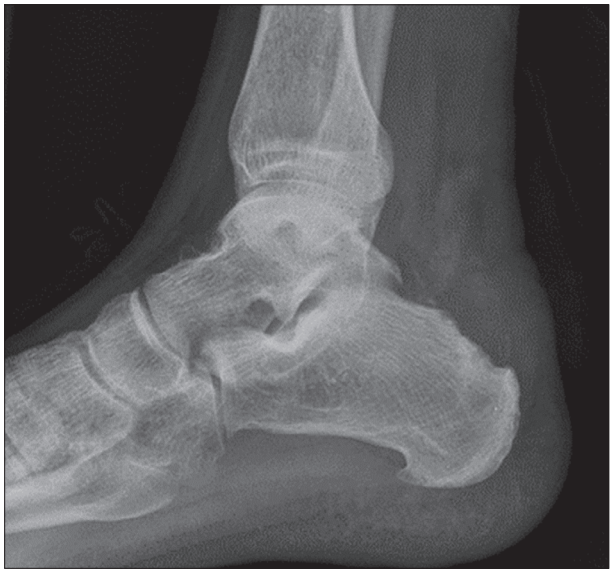

Figure 3. Initial postoperative X-ray of the heel bone

RESULTS

The surgeries were all performed by the same surgeon, having first own experience in this type of the treatment. The average operation time was 60 minutes (40–90 minutes). All wounds healed normally, and, due to a minimal surgical trauma, the rehabilitation was accelerated. Postoperative x-rays showed a satisfactory degree of resection in most cases. In all patients, a significant improvement and reduction of symptoms were noted in the early phase of rehabilitation. There were no complications. Three patients, started with full weight-bearing and sports activities too early, had occasional pain and swelling up to the third month after surgery.

The AOFAS score showed an improvement. The average values were 59.3 ± 11.48 preoperatively, and 90.1 ± 7.55 confirming a significant change ($p < 0.005$).

DISCUSSION

Haglund's syndrome is initially treated conservatively. This includes application of nonsteroidal anti-inflammatory drugs, use of wide shoes, heel lifts, change of activity, calf muscle stretching exercises, and other physical therapy procedures. There is also a description of the local use of corticosteroid injections, where special attention should be paid to the risk of damage to the Achilles tendon [13]. According to the literature, the outcome of conservative treatment is unpredictable. Myerson and McGarvey [14] reported a good result in 85% of non-surgically treated patients. On the other hand, Sammarco and Taylor [15] reported a considerably lower success in non-surgical treatment, with as high as 65% of unsuccessful outcomes. If non-surgical treatment lasts longer than six months with persisting problems, than surgical treatment is considered.

During the last two decades, there have been significant changes to the surgical treatment of Haglund's syndrome. Open surgery with the removal of a retrocalcaneal bursa and remodeling of the heel bone showed good results in 50–100% of cases [5, 15]. Open surgery with wedge osteotomy of the calcaneus is sometimes still used [16]. However, these types of surgery were accompanied by some complications, including wound dehiscence, infection, Achilles tendon rupture, painful surgical scar, and limited ankle motion [5, 17, 18]. Ehredt et al. [19] described a combination of endoscopic calcaneoplasty and gastrocnemius resection, but they reported no data about its safety and efficacy in the treatment of Haglund's syndrome.

Endoscopic surgery has advantages over an open approach: smaller surgical wound, less tissue trauma, better visualization of the pathological process itself, faster recovery, minimal complications from wound healing, and minimal surgical scar [8, 20]. Today there is a growing trend of endoscopy over an open joint surgery, due to the lower rate of wound healing problems, as well as much faster rehabilitation [21, 22]. This problem is common among professional athletes, for whom the period of absence from the field is very important. After an open surgery of Haglund's syndrome, the period of return to the field lasts up to nine months, while it lasts from three to six months after an endoscopic intervention [4, 7, 22].

Our study confirmed good results. There were no complications regarding surgical wounds. The three-portal technique was used, offering better visualization than the two-portals technique. This approach provides a good, direct view of the Achilles tendon and its attachment allowing the removal of the hypertrophic retrocalcaneal bursa and the bone prominence. Bone resection was minimal but sufficient and it was controlled intraoperatively by the checks whether impingement occurs. The operation time was shorter at each subsequent operation. There were no major pain and swelling in the early postoperative period, and all patients completed the rehabilitation according to the protocol. Three patients were professional athletes, thus they were interested for as quick return to the field. As they did not have major problems, they started slightly earlier with a higher load than other patients, which led to swelling and pain. The symptoms decreased with analgesic therapy and rest. Our opinion is that patients with the need for greater physical activities should wait for that at least three months. The patients were generally satisfied with the final outcome, which was confirmed by the AOFAS score results.

CONCLUSION

The endoscopic approach with the three-portal technique in Haglund's deformity removal is expected to give a good result. Its advantages include a quick return to regular activities, followed by minimal complications. The procedure requires endoscopic instruments, adequate surgical technique, and an educated surgeon.

Conflict of interest: None declared.

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Предности ендоскопске хирургије у лечењу Хаглундовог синдрома употребом технике са три портала

Миодраг Глишић[1,2], Владан Стевановић[1,2], Александар Јевтић[1,2], Милан Мирковић[1], Љубомир Даковић[1], Анхелика Арсе Станковић[1], Сања Мирковић[3], Лазар Мичета[1,2]

[1]Институт за ортопедију „Бањица", Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија;
[3]Универзитет у Београду, Факултет спорта и физичког васпитања, Београд, Србија

САЖЕТАК

Увод/Циљ Деформитет пете праћен болом на месту припоја Ахилове тетиве познат је као Хаглундов синдром. Проминенција суперолатералног дела петне кости која врши притисак на ретрокалканеалну бурсу и Ахилову тетиву доводи до отока и бола. Само стање се може лечити како неоперативно, тако и оперативно.

Приказали смо прва искуства у ендоскопском решавању Хаглундовог синдрома коришћењем технике са три портала.

Методе У периоду од јануара 2019. до маја 2020. године учињено је 10 ендоскопских интервенција ремоделације петне кости са ресекцијом ретрокалканеалне бурзе. Дијагноза је постављена на основу анамнезе, клиничког прегледа, рендгенских снимака и магнетне резонанце. За процену резултата коришћен је резултат теста Америчког ортопедског друштва за стопала и скочни зглоб (енг. *American Orthopedic Foot and Ankle Society*) пре операције и шест месеци од оперативног лечења.

Резултати Ендоскопски налаз је код свих пацијената показао хипертрофичну ретрокалканеалну бурзу и проминенцију на задњем горњем делу петне кости који прави притисак на Ахилову тетиву. Коришћењем технике са три портала добија се много боља визуализација и лако се уклања измењено ткиво. Радиографска контролна слика је била задовољавајућа. Резултат скора Америчког ортопедског друштва за стопала и скочни зглоб показује значајно побољшање после ове хируршке интервенције.

Закључак Ендоскопски приступ решавању Хаглундовог синдрома са три портала је сигурна процедура која даје добре резултате. Омогућује бржи опоравак уз мање компликације у односу на отворену хирургију.

Кључне речи: ендоскопска калканеопластика; калканеус; Хаглундов синдром; ретрокалканеални бурзитис

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Pulmonary air leak syndrome in a premature infant born to mother with COVID-19

Lidija Banjac[1,2], Boban Banjac[2]

[1]Clinical Center of Montenegro, Institute of Children's Diseases, Department of Neonatology, Podgorica, Montenegro;
[2]University of Montenegro, Faculty of Medicine, Podgorica, Montenegro

SUMMARY
Introduction The clinical course of premature infants born to mothers with coronavirus disease 2019 (COVID-19) has not been well characterized. The aim of this paper was to report a complicated clinical course of pulmonary air leak syndrome (pneumomediastinum and pneumothorax) in a premature infant born to a mother with COVID-19.
Case outline The patient was a male infant born at 35 weeks of gestation. The mother had confirmed coronavirus pneumonia six days prior to delivery. At approximately 25 hours of age, chest X-ray showed pneumomediastinum, giving the classic "spinnaker-sail" sign. After intubation, chest X-ray showed the typical "angel-wing" sign, which indicates pneumomediastinum and bilateral pneumothorax (pulmonary air leak syndrome).
Conclusion Based on the presented case, we believe that the mother's COVID-19 infection is an additional risk factor for the occurrence of pulmonary air leaks in the infant. To confirm this hypothesis as well as explain the exact pathophysiology of air leakage in COVID-19, larger, prospective, and well designed studies are needed.
Keywords: coronavirus disease 2019; premature infants; pulmonary air leak syndrome; pneumomediastinum; pneumothorax

INTRODUCTION

Coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is a highly contagious and potentially life-threatening disease. The COVID-19 pandemic has been declared by the World Health Organization (on March 11th, 2020) as a global public health emergency [1]. The disease is mild in most people, but in some it may progress to pneumonia, acute respiratory distress syndrome, and multiorgan dysfunction. Disease in infants and children has also been reported to be significantly milder than in adults. Mortality rates range 0–12% in the pediatric population [2].

The published data on premature infants born to mothers with COVID-19 has been limited. Studies had no evidence to suggest that the development of COVID-19 pneumonia in the third trimester of pregnancy could lead to the occurrence of severe adverse outcomes in infants [3, 4]. But some studies describe a very complicated clinical course with pulmonary air leak syndrome in preterm infants born to mothers with COVID-19. The management of these infants presents a significant challenge [5, 6].

The aim of this paper was to report a very complicated clinical course of pulmonary air leak syndrome in a preterm infant born to a mother with COVID-19.

CASE REPORT

A patient was a male infant born at 35 weeks of gestation with birth weight of 2600 g. He was born to a 31-year-old (gravida 2, para 2) who had received treatment for hypothyroidism during pregnancy.

Maternal history was significant for cough and fever six days prior to delivery. She was admitted to the hospital one day prior to delivery and tested positive for SARS-CoV-2 on reverse-transcription polymerase chain reaction (RT-PCR) by a nasopharyngeal swab. After chest X-ray, she was diagnosed with COVID-19 pneumonia. Laboratory findings showed a high level of C-reactive protein (61 mg/L) and a slightly lower white blood cell count (5.6×10^9/L) Caesarian section was performed at 35 weeks of gestation after premature rupture of membranes. At the time of delivery, she was on high-flow nasal cannula oxygen therapy and prophylactic anticoagulation with low-molecular-weight heparin due to the risk of thrombosis in pregnancy and COVID-19.

The patient (male premature infant) was vigorous at birth, Apgar scores were 8 at both one and five minutes, and he required only the fraction of inspired oxygen (FiO_2) of 0.3. The infant was immediately separated from the mother, transferred to the Pediatric hospital, and admitted to an isolation room due to maternal COVID-19. Initial chest X-ray was suggestive of respiratory distress (Figure 1A), and

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Correspondence to:
Lidija BANJAC
Department of Neonatology
Clinical Center of Montenegro
10 Ljubljanska Street
Podgorica 81000, Montenegro
drbanjac1@gmail.com

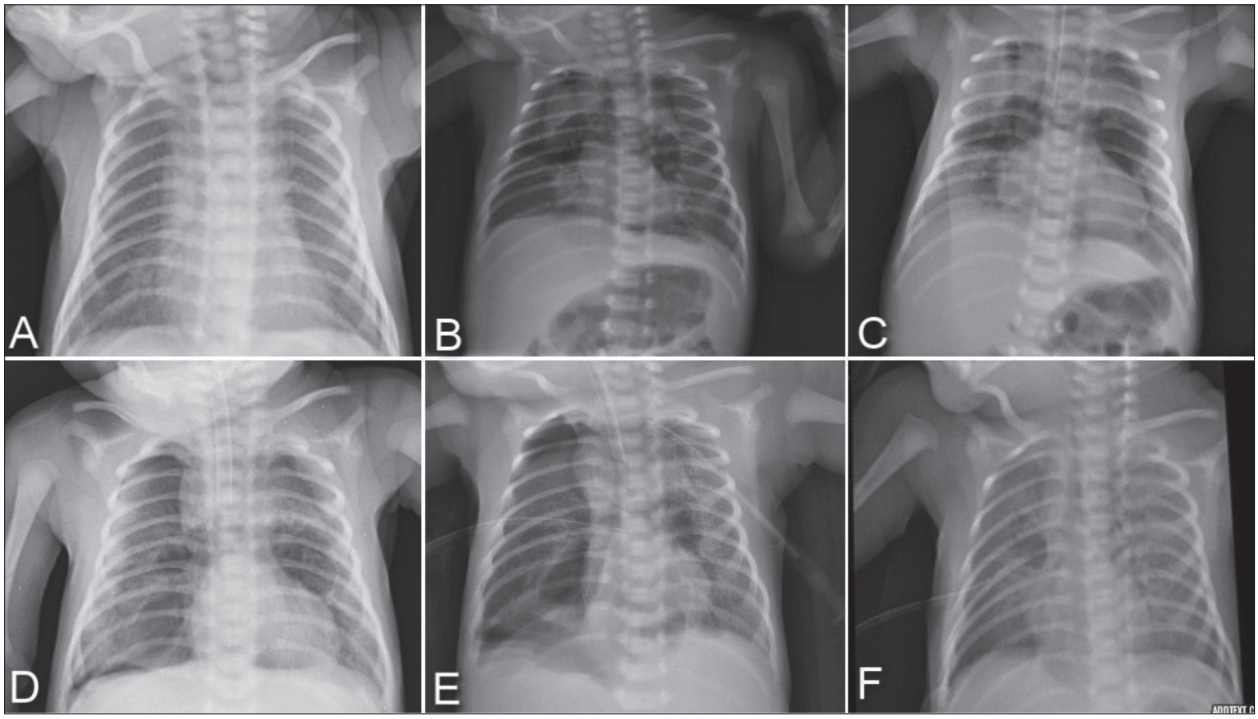

Figure 1. Chest radiographs; on admission, a chest X-ray showed granular lungs with air bronchograms in the central regions (A); after worsening health conditions, chest X-ray showed unusual pneumomediastinum – a central area of radiolucency projected over the mediastinum, giving the classic "spinnaker-sail" sign (B before and C after intubation); chest X-ray after seven hours showed the typical "angel-wing" sign – bilateral hyperlucency representing air leak into the pleural cavities – pneumothoraces (D), which required bilateral thoracic drainage followed by chest tube placement (E); a repeat chest X-ray was performed at the age of nine days, which showed partial resolution of the pneumomediastinum and gradual improvement of the patient's symptoms; the patient was extubated (F)

his initial capillary blood gas showed a pH of 7.3, PCO_2 of 4.6, PO_2 of 4.8, and base excess of -6.2. The infant was started on caffeine prophylaxis for apnea of prematurity and empiric antibiotics (ampicillin and gentamicin) due to preterm labor. He was tested negative for SARS-CoV-2 on RT-PCR by nasopharyngeal swab.

A few hours after the birth, the infant developed increased work of breathing; thus, he was transferred to the neonatal intensive care unit and nasal continuous positive airway pressure respiratory support was commenced.

At approximately 25 hours of age, the infant had desaturations requiring an increase in oxygen supplementation, and chest X-ray showed an unusual radiographic finding of pneumomediastinum – a central area of radiolucency projected over the mediastinum separating the thymic lobes upwards and outwards, giving the classic "spinnaker-sail sign" (also known as a "angel-wing sign") (Figure 1B). Because of respiratory deterioration, the infant was intubated. Chest X-ray after intubation revealed pneumomediastinum and bilateral pneumothorax requiring thoracic drainage (Figures 1C, 1D, and 1E).

Mechanical ventilation with a positive end-expiratory pressure of 6 cm of H_2O, FiO_2 of 0.6, and a mean airway pressure of 22 cm of H_2O was started. Exogenous surfactant instillation resulted with an improvement in oxygen requirement.

Antibiotics were replaced to meropenem and amikacin, and were discontinued after 72 hours, upon the report of negative cultures.

The follow-up chest X-ray showed an incomplete resolution of air leaks (Figure 1F).

The infant's respiratory function improved, he was extubated on the eighth hospital day, and chest tubes were removed the next day.

The need for supplementary oxygen therapy was decreased, and the infant was placed in a crib, bottle-fed, and discharged from the hospital seven days after extubation. No air leakages were detected in a chest X-ray performed before hospital discharge.

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient/parent/guardian for the publication of this case report and any accompanying images.

DISCUSSION

COVID-19 is a highly contagious and potentially life-threatening disease, especially in pregnancy [7]. The published data on premature infants born to mothers with COVID-19 has been limited [7, 8]. It is reliably known that the mother's COVID-19 infection leads to preterm delivery, but the connection to the occurrence of pulmonary air leak in the newborn has not been fully proven. Pulmonary air leaks in infants are certain to be associated with high morbidity and mortality [7, 9, 10].

This case report shows the clinical course of a premature infant born to a mother with COVID-19 pneumonia.

He had initial mild (moderate) respiratory distress complicated by the development of severe pulmonary air leak syndrome (pneumomediastinum, bilateral pneumothorax) with unusual chest X-ray signs ("spinnaker-sail" or "angel-wing" sign).

The infant had some risk factors for air leak: male sex, late preterm gestation, and cesarean delivery predispose him to transitory tachypnea, which leads to pulmonary air leak. Considering the severity of our patient's clinical condition, we believe that COVID-19 maternal pneumonia in the third trimester of pregnancy was an additional risk factor for preterm delivery, caesarean section, and pulmonary air leak syndrome in infants.

Our opinion is supported by published studies on term and preterm infants from COVID-19-positive mothers with pulmonary air leak syndrome, as well as the fact that pneumomediastinum is considered the leading cause of maternal death [11]. Recent review studies reveal a high incidence of air leaks in patients with COVID-19, even in the absence of any traditional risk factors [12].

Although the precise pathophysiology of air leaks with COVID-19 is still unknown, some studies suggest that a variety of factors may contribute to its development [12, 13]. According to Harancang et al. [14], COVID-19 starts an inflammatory dysregulation that results in diffuse damage and alveolar rupture.

On the other hand, several studies did not suggest that the development of COVID-19 pneumonia in the third trimester of pregnancy could lead to the occurrence of severe adverse outcomes in infants [2, 3, 15].

A national prospective epidemiological study that included all infants with COVID-19 in Spain (40 cases) showed that clinical manifestations were mild, such as upper respiratory airway infections, febrile syndrome, acute gastroenteritis, apnea, and mild respiratory distress. The most severe manifestations occurred in two preterm infants with pneumonia and in an infant with bronchiolitis due to rhinovirus co-infection. Respiratory support was required in several cases, and in those who did need it, oxygen and non-invasive systems were briefly used [6].

Anand et al. [16] described 65 infants born to COVID-19-positive mothers. Approximately one-third of the cohort was born preterm (40%). Of the 65 infants, seven tested positive for COVID-19. Six of the seven infants were asymptomatic, and one infant received respiratory support (CPAP) for 48 hours.

Also, very complicated neonatal illness with pulmonary air leak syndrome was described in preterms born to mothers with COVID-19. The management of these infants presents a significant challenge [4, 5].

Piersigilli et al. [5] describe a female infant at 26 gestational weeks born to a mother who developed COVID-19 positive bilateral pneumonia. Despite the initial clinical stability and the requirement of low FiO_2, 12 hours after the less invasive surfactant administration procedure, she developed a pneumothorax requiring thoracic drainage. In our patient, air leak developed before surfactant administration, which excludes surfactant as an etiological factor.

Reddy and associates presented two preterm infants born to mothers with COVID-19, who also developed acute onset severe air leak syndrome requiring thoracic drainage [4].

In another study by Kamity et al. [17], they assumed that *in utero* exposure to SARS-CoV-2 leads to fetal pneumonitis, which is the reason for the increased susceptibility to pneumothorax. They advise increased vigilance in infants born to COVID-19-positive mothers even when their SARS-CoV-2 PCR is negative.

Zhu et al. [18], in a clinical analysis of 10 infants born to mothers with COVID-19 pneumonia, concluded that perinatal COVID-19 may have adverse effects on infants, causing problems such as premature labor, respiratory distress, and even death. Seven out of nine infants had abnormal chest computer tomography with a picture of neonatal respiratory distress syndrome, infection, and pneumothorax.

Wróblewska-Seniuk et al. [13] concluded that higher respiratory distress rates and the need for respiratory support in infants resulted only from COVID-19 infection in the mother. However, according to the authors, the mechanism of pulmonary air leak is not easily explicable.

Despite the increasing amount of published data on COVID-19 in pregnancy, there is insufficient good-quality data to draw unbiased conclusions regarding the influence of maternal COVID-19 infection on the occurrence of pulmonary air leak in infants [19].

We believe, based on our case, that COVID-19 infection in the mother is an additional risk factor for the development of pulmonary air leak in the infant. This statement is supported by the high incidence of pneumomediastinum in patients with COVID-19 infection and the fact that pneumomediastinum is the most common cause of death in pregnant women with COVID-19 [11, 12, 14].

To confirm this hypothesis as well as explain the exact pathophysiology of air leakage in COVID-19, larger, prospective, and well-designed studies are needed.

In accordance with the obtained data, the guidelines for neonatal care and treatment, especially respiratory support, should be revised. In any case, we recommend special attention to prevent COVID-19, systematic screening of suspected infections during pregnancy, and extended intensive follow-up for infants, especially preterm infants.

Conflict of interest: None declared.

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Плућни синдром цурења ваздуха код претерминског новорођенчета рођеног од мајке са ковидом 19

Лидија Бањац[1,2], Бобан Бањац[2]

[1]Клинички центар Црне Горе, Институт за болести дјеце, Центар за неонатологију, Подгорица, Црна Гора;
[2]Универзитет Црне Горе, Медицински факултет, Подгорица, Црна Гора

САЖЕТАК

Увод Клинички ток претерминске новорођенчади рођене од мајке са ковидом 19 није довољно испитан.

Циљ овог рада је био да прикаже претерминско новорођенче са плућним синдромом цурења ваздуха (пнеумомедијастинум и пнеумоторакс) рођеног од мајке позитивне на ковид 19.

Преглед болесника Болесник је мушко новорођенче рођено у 35. недељи гестације. Мајка је шест дана пре порођаја имала коронавирусну упалу плућа. Радиографија грудног коша новорођенчета у 25. сату по рођењу показала је необичајени пнеумомедијастинум – знак „троугластог једра". Након интубације, на радиографији грудног коша приказује се „знак анђеоских крила", који представља пнеумомедијастинум и билатерални пнеумоторакс (плућни синдром цурења ваздуха).

Закључак На основу приказаног случаја, сматрамо да је коронавирусна болест мајке додатни фактор ризика за појаву плућног синдрома цурења ваздуха код новорођенчета. За потврду ове хипотезе, као и за објашњења тачне патофизиологије цурења ваздуха код ковида 19, потребне су веће, проспективне и добро осмишљене студије.

Кључне речи: ковид 19; претерминско новорођенче; плућни синдром цурења ваздуха; пнеумомедијастинум; пнеумоторакс

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Rare case of myelodysplastic syndrome with near-tetraploidy and *TP53* mutation

Nataša Čolović[1,2], Vesna Đorđević[1], Milica Radojković[2,3], Teodora Karan-Đurašević[4], Nataša Tošić[4]

[1]University Clinical Center of Serbia, Clinic of Hematology, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[3]Dr. Dragiša Mišović Clinical Hospital Center, Clinic of Internal Medicine, Belgrade, Serbia;
[4]University of Belgrade, Institute of Molecular Genetics and Genetic Engineering, Belgrade, Serbia

SUMMARY

Introduction Chromosomal numerical aberrations are very common in hematological malignancies, but near-tetraploidy (80–104 chromosomes) is rare in myeloid lineage malignancies, with only a few cases reported in myelodysplastic syndrome (MDS). Due to a small number of cases with this rare cytogenetic abnormality, clinicopathological significance of near-tetraploidy in MDS is still unknown. In this case report we present a case of *de novo* MDS patient with near-tetraploidy in association with *TP53* mutation, and we aimed to elucidate the prognostic significance of this rare genetic feature.

Case outline In August of 2018, a 71-year-old male presented with severe anemia, thrombocytopenia, leucopenia, and enlarged spleen. Laboratory data were as follows: hemoglobin (Hb) 93 g/L, white blood cells 2.8×10^9/L and platelets 23×10^9/L. The bone marrow aspirate was hypercellular, megakaryocytes were not found, 15% of granulocytic cells were with signs of dysplasia, and 16% of blast cells without Auer rods. The finding was in correlation with diagnosis of MDS, type refractory anemia with excess blasts 2 which was also confirmed by immunophenotyping. Cytogenetic finding was near-tetraploidy (48,XY+mar[10]/92,XXYY[10]), and *TP53* mutational analysis showed the presence of mutation in exon 8 (p.D281A; c.842 A > C). The patient received from time to time packed red blood cells and platelets, and died four months after initial diagnosis.

Conclusion Near-tetraploidy associated with *TP53* mutation has been described in only a few MDS cases. Results of these reports including ours suggest that the association of *TP53* mutation and near-tetra polyploidy is a poor prognostic factor.

Keywords: near-tetraploidy; *TP53* mutation; myelodysplastic syndrome; prognosis

INTRODUCTION

Myelodysplastic syndromes (MDS) are a group of clonal hematopoietic stem cell malignancies characterized by ineffective hematopoiesis, bone marrow dysplasia, peripheral blood cytopenia and by intrinsic risk of acute myeloid leukemia (AML) transformation [1]. Chromosomal abnormalities may be numeric and structural and can be found in about 50% of primary MDS and in around 80% patients with secondary MDS after chemotherapy or some toxic agents [2, 3]. Chromosomal abnormalities can vary from a single chromosome abnormality such as monosomy, to a complex karyotype. Numerical abnormality like near-tetraploidy (80–104 chromosomes) is rare in myeloid lineage hematologic malignancies like MDS and it is associated with poor outcome [4]. In addition to pretreatment karyotype being essential for risk stratification and treatment of MDS patients, in recent years the influence of mutations detected in over 89% of cases, has been making its impact on the prognostic stratification model [5]. Mutations in *TP53* gene detected in around 10% of novel MDS cases has been shown to have independent adverse prognostic effect [6].

Here, we report the case of a 71-year-old man diagnosed with MDS, with near-tetraploidy accompanied with *TP53* mutation.

CASE REPORT

In August 2018, a 71-year-old man, with a history of diabetes mellitus and hypertension, in presented with severe anemia, thrombocytopenia, leucopenia, and enlarged spleen with diameter 159 × 64 mm on ultrasonography. He was admitted at the University Clinical Center of Serbia, Clinic of hematology on under the suspicion of evolution of MDS in AML, Eastern Cooperative Oncology Group performance status 2, and Hematopoietic Cell Transplantation-Comorbidity Index 1.

Laboratory findings were: hemoglobin (Hb) 93 g/L, white blood cells (WBC) 2.8×10^9/L, platelets 23×10^9/L (leukocyte formula: segmented 11%, lymphocytes 70%, monocytes 2%, eosinophils 3%, basophils 11%, metamyelocytes 1%, blasts 2%, erythroblasts 7/100 WBC). Biochemical analyses were: glycaemia 9.3 mmol/l, total bilirubin 33 μmol/L, ferritin 547 ng/ml, fibrinogen 5.87 g/L, d-dimer 1.29 mg/L. Virology, human immunodeficiency virus, hepatitis B surface antigen and hepatitis C were negative.

The bone marrow aspirate was hypercellular, megakaryocytes were not found, granulocytic cells were 15% with signs of dysplasia, hypogranular and hyposegmented Pelgeroid-like neutrophil element, with 16% of blast cells,

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Correspondence to:
Nataša TOŠIĆ
University of Belgrade
Institute of Molecular Genetics and Genetic Engineering
Laboratory for Molecular Biomedicine
Vojvode Stepe 444a
11042 Belgrade
Serbia
natasa.tosic@imgge.bg.ac.rs

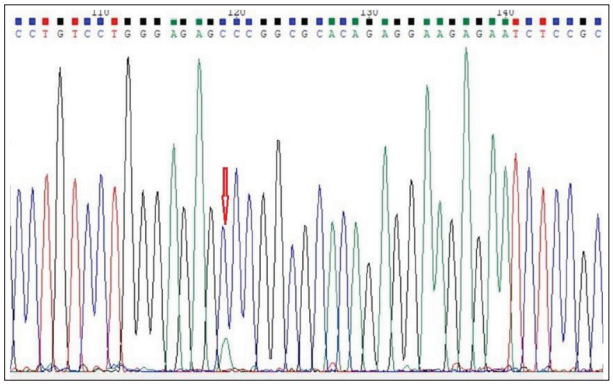

Figure 1. Direct sequencing of exon 8 of the*TP53* gen amplified by polymerase chain reaction; the red arrow shows heterozygous, missense mutation at the position 842 (A-green, to C-blue; c.842A > C), resulting in the substitution of amino-acid at the position 281 (Asp to Ala; p.D281A); it has been reported in the COSMIC (Catalogue of somatic mutations in cancer) data base by number COSM11665

without Auer rods, 40% of blast cells were myeloperoxidase positive, erythroid cell line 62% striking was megaloblastic, with presence of two to three nucleoli in erythroblasts with signs of vacuolization. The finding was in correlation with diagnosis of MDS, type refractory anemia with excess blasts (RAEB 2). Immunophenotyping of bone marrow cells done by flow cytometry, showed positivity for HLA-DR, CD34, CD71, CD38, CD200, CD123, cMPO, clizozime, CD117, CD3, CD22. This results also correlated with diagnosis of RAEB 2.

Cytogenetic finding was 48,XY+mar[10]/92,XXYY[10]. Molecular analyses of *SF3B1* and *TP53* gene was done using polymerase chain reaction followed by direct sequencing [7, 8]. The patient was *SFB3B1* negative, but in *TP53* gene we detected a single mutation in exon 8 (p.D281A; c.842 A > C) (Figure 1). The diagnosis of MDS, type RAEB 2 was confirmed with Eastern Cooperative Oncology Group performance status 2 and Hematopoietic Cell Transplantation-Comorbidity Index 1. The patient was unwilling to undergo intensive treatment with chemotherapy. In further course he received from time to time packed red blood cells and platelets. He died in December 2018.

All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

DISCUSSION

MDS is a highly heterogeneous group of disorders with numerous genetic aberrations. Cytogenetic findings are important prognostic factor incorporated in almost all prognostic scoring systems. Presence of three or more chromosomal anomalies is regarded as complex karyotype which can be associated with progression of MDS to AML. Comparing the karyotype in acute leukemias and MDS, numeric aberrations dominate in MDS while in acute leukemias structural aberrations are dominant. Balanced cytogenetic abnormalities, including reciprocal translocations, inversions and insertions, are prevalent in myeloid leukemias but are uncommon in MDS, in which unbalanced numeric chromosomal abnormalities reflecting a gain or loss of chromosomal material are more prevalent [3].

Numeric chromosomal abnormality near-tetraploidy could be found in 1.2% of AML, but only in 0.57% of MDS patients [9, 10]. Tumor suppressor gene *TP53* is located on the short arm of chromosome 17(17p13) [9]. *TP53* gene encodes p53 protein which is main regulator of cellular homeostasis, cellular division, DNA-damage replication and apoptosis [9, 11]. *TP53* overexpression may precede to a change of diploidy to tetraploidy state of the cell population, enabling DNA duplication without cell division leading to polyploidia. Near-tetraploidy associated with *TP53* mutation has been described only in four MDS cases [9]. Haase at al. [12] analyzed cytogenetic findings in a cohort of 2072 patients with MDS but there was no one patient with near-triploidy or near-tetraploidy karyotype. In a study on 1576 patients with MDS the incidence of near-triploidy and near-tetraploidy was 0.57% [12]. In this cohort study, the authors have found nine patients with near-triploidy and near tetraploidy karyotype, but association with *TP53* mutation is not described. Among them, eight had only polyploidy, without other aberrations and one had at the same time complex karyotype. In the group of 979 adult patients with different hematological malignancies, association of *TP53* mutation and near-triploidy or near-tetraploidy karyotype was diagnosed in four MDS patients, three with RAEB and one with refractory cytopenia with multilineage dysplasia (RCMD). Patients with RAEB lived two, three, and six months while patient with RCMD lived 18 months.

In conclusion, *TP53* mutation are found in 5–20% patients with MDS, more frequent in high-risk group associated with complex karyotype involving chromosome 5, 7, and 17 causing negative impact on prognosis [13, 14]. However, *TP53* mutations are rarely associated with near-triploidy or near-tetraploidy karyotype. Latest research of MDS genomic landscape showed that *TP53* mutations are frequently associated with aneuploidy and chromothripsis, and not with other MDS "driver" mutations, suggesting that for *TP53* mutations, alterations at chromosome level represent cooperating, "second hit" event, driving MDS towards leukemic transformation [15]. In spite of the small number of reported cases, it is impossible to determine the prognostic impact of the combined occurrence of near-tetraploidy and *TP53*mutation. Based on our case report, we could speculate that it has a poor impact on the prognosis and outcome of the disease.

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Мијелодиспластични синдром са приближном тетраплоидијом удруженом са мутацијом гена *TP53* – редак случај

Наташа Чоловић[1,2], Весна Ђорђевић[1], Милица Радојковић[2,3], Теодора Каран-Ђурашевић[4], Наташа Тошић[4]

[1]Универзитетски клинички центар Србије, Клиника за хематологију, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија;
[3]Клиничко-болнички центар „Др Драгиша Мишовић", Клиника за интерну медицину, Београд, Србија;
[4]Универзитет у Београду, Институт за молекуларну генетику и генетско инжењерство, Београд, Србија

САЖЕТАК

Увод Нумеричке аберације хромозома су веома честе код хематолошких малигнитета, али су приближне тетраплоидије (80–104 хромозома) ретке у малигнитетима мијелоидне лозе, са само неколико случајева пријављених у мијелодиспластичком синдрому (МДС). Због малог броја случајева са овом ретком цитогенетском абнормалношћу, клиничко-патолошки значај приближне тетраплоидије у МДС-у је још увек непознат. Овим приказом *de novo* болесника са МДС-ом, са приближном тетраплоидијом и мутацијом у гену *TP53*, циљ нам је био да расветлимо прогностички значај ове ретке генетске карактеристике.

Приказ болесника Приказан је 71-годишњи болесник који је у августу 2018. године развио симптоме тешке анемије, тромбоцитопеније, леукопеније и увећане слезине. Лабораторијске анализе су показале следеће: хемоглобин 93 g/L, леукоцити $2,8 \times 10^9$/L и тромбоцити 23×10^9/L. Аспират коштане сржи је био хиперћелијски, мегакариоцити нису нађени, 15% гранулоцита је било са знацима дисплазије, 16% бласта без Ауерових штапића. Налаз је одговарао дијагнози МДС-а, типа рефракторне анемије са вишком бласта 2, што је потврђено и имунолошком фенотипизацијом. Цитогенетском анализом утврђено је присуство приближне тетраплоидије (48,*XY*+*mar*[10]/92,*XXYY*[10]), а анализа мутација у гену *TP53* показала је присуство мутације у егзону 8 (*p.D*281*A*; *c.*842 *A* > *C*). Болесник је по потреби примао трансфузију еритроцита и тромбоците, а умро је четири месеца након почетне дијагнозе.

Закључак Присуство приближне тетраплоидије удружене са мутацијама у гену *TP53* описано је само у неколико случајева МДС-а. Резултати ових случајева, као и наши резултати, указују на то да приближна тетраплоидија повезана са присуством мутација у гену *TP53* представља фактор лоше прогнозе.

Кључне речи: приближна тетраплоидија; мутације гена *TP53*; мијелодиспластични синдром; прогноза

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Challenges in surgery of deep burns

Biljana Ćertić[1], Anđela Dimkić-Milenković[1], Milan Jovanović[1,2]

[1]University Clinical Center of Serbia, Clinic for Burns, Plastic and Reconstructive Surgery, Belgrade, Serbia; [2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

SUMMARY

Introduction Full-thickness burns pose a significant challenge in terms of surgical management, particularly when concurrent trauma of other organs is involved. Traditional treatment of deep burns includes early excision or debridement of necrotic tissue, followed by skin grafting or flap reconstruction. There are numerous challenges such as poor overall general condition, polytrauma, questionable wound bed viability, limited donor sites. Thus, we have to consider skin substitutes. INTEGRA® is an acellular dermal substitute which creates a native dermis. The aim of this case was to share our experience of the treatment by skin substitutes in a polytraumatized burn patient.

Case outline We present a case report of a 46-year-old man with severe work-related contact burn wounds associated with multiple rib and vertebral fractures, as well as lungs contusion with localized bilateral hemothorax. Patient suffered from third-degree burns to the lower extremities, extending to scrotal and gluteal area, which included 15% of the total body surface area. The patient underwent early excision of necrotic tissues with subsequent skin autografting on the right leg; however, due to partial failure of autografts, we had to perform allografting followed by autografting because of limited local donor sites and poor general condition. Successive debridement and partial osteotomy resulted in the left knee defect with exposed patella. Therefore, in order to reconstruct the consequent defect and prevent joint contracture, the defect was finally covered by INTEGRA®.

Conclusion Our experience has highlighted that INTEGRA® prevents functional disability and furthermore, it leads to optimal aesthetic results.

Keywords: INTEGRA®; full-thickness burns; exposed bone; skin grafts; reconstruction

INTRODUCTION

Understanding of the pathophysiological abnormalities occurring not only locally but also systematically after burn injury is essential and leads to optimal treatment of burn patients [1]. Full-thickness burns pose a significant challenge in terms of surgical management in modern burn care [2]. Since burn illness may be greatly complicated by the persistence of an open wound due to malnutrition and bacterial invasion, the wound must be promptly closed. It would be of great importance to reduce the severity of hypertrophic scarring, postburn contractures, as well as promote faster rehabilitation [3]. Therefore, as soon as the overall status of the patient permits it, full-thickness burns should be prepared for debridement followed by autografting or flap reconstruction. However, these options may not be suitable for every patient. There are numerous challenges, which limit standard methods of repair, such as concurrent trauma of other organs, poor general condition, questionable underlying wound bed viability, limited donor sites; thus, we have to consider skin substitutes. Skin substitutes remain a fundamental part of the burn therapy system. They vary from skin allografts over xenografts to the dermal matrix [4]. Their common role is to overcome these challenges, with the greatest possible functional and aesthetic outcomes [5].

The aim of this report was to share our experience of the treatment by skin substitutes in the polytraumatized burn patient.

CASE REPORT

We present a case report of a 46-year-old man whose both legs, gluteal, and scrotal area were crushed by a glowing-hot metal construction at his work place.

Initially, the patient was referred to the Emergency Center under polytrauma alert, where he was examined by a neurosurgeon, an orthopedic surgeon, a thoracic surgeon, and an anesthesiologist. An X-ray of the thorax, ultrasound of abdomen, and multidetector computed tomography of the cervical spine revealed evidence of multiple rib and vertebral fractures, as well as lungs' contusion with localized bilateral hemothorax.

Due to the nature of the injuries, the patient was admitted and evaluated at our clinic 6.5 hours after the accident. The patient suffered third-degree contact burns with total burned body surface area of 15%, including the lower extremities, gluteal, and scrotal area (Figures 1A and 1B). At the fifth posttraumatic day, after bilateral thoracentesis, which had to be performed, patient underwent surgical debridement, followed by autografting on his right leg to the level of the fascia; the left thigh served as

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Correspondence to:
Anđela DIMKIĆ MILENKOVIĆ
Gramšijeva 9/45
11070 Novi Beograd, Serbia
adimkic@yahoo.com

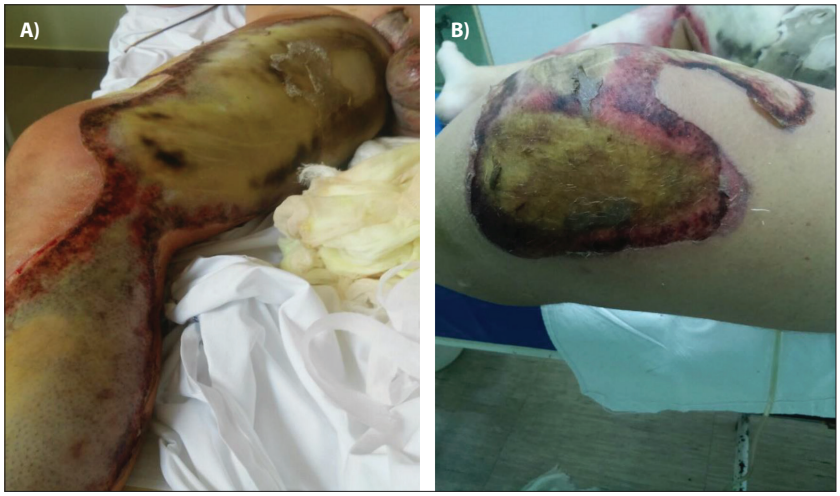

Figure 1. Local status on admission: third-degree burns to the lower extremities, scrotal, and gluteal area, which covered 15% of the total body surface area; patient on admission: A) right leg; B) left knee

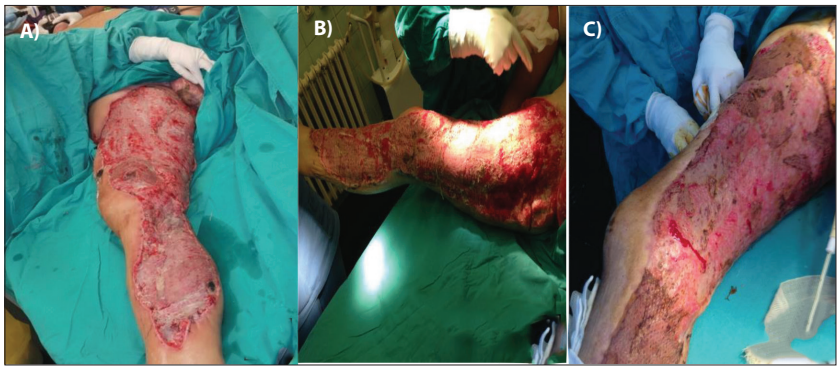

Figure 2. A: Fifth post-traumatic day – the right lower extremity after autografting; B: 12th day after autografting; partial failure of autografts; we performed allografting with fresh donor skin; C: status post allografting followed by autografting

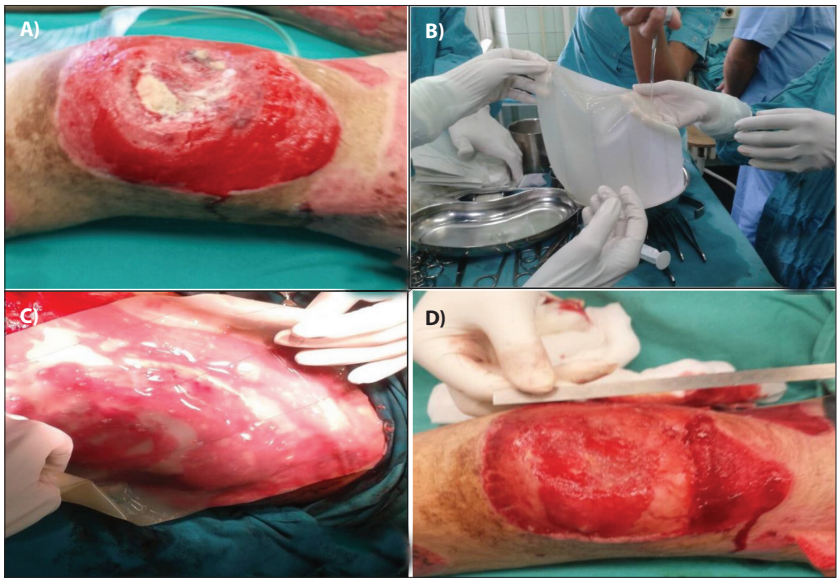

Figure 3. A: Radical debridements and partial ostectomy resulted in the left knee defect with exposed patella; B: in the preoperative planning, an INTEGRA patch of 20 × 15 cm in size was selected; C: placement of the INTEGRA matrix; the defect of the left knee that was managed with the INTEGRA matrix; D: status – post INTEGRA matrix placement; 18th day after initial INTEGRA matrix placement; the INTEGRA matrix had incorporated; treated area led to neodermis formation, which measured 22 × 18 cm

the donor site (Figure 2). Twelve days after autografting, due to partial failure of the autografts, we had to perform allografting, because of poor general condition and the limitation of the local donor site (Figure 2A). On the 23rd hospitalization day, autografting was performed again (Figure 2B), and the result was stable epithelium on the right lower extremity. Dressing changes were performed throughout treatment. Scrotal and gluteal areas were successfully reconstructed by autografting.

Furthermore, successive debridements and partial otectomy resulted in the left knee soft tissue defect with exposed patella (Figure 3A).

Our patient was not a candidate for flap reconstruction, because there were scars from previous donor sites. Therefore, we considered dermal replacement matrices in order to augment and improve the regeneration of the dermis. After 30 days, anterior part of the left knee after osteotomy was covered by the INTEGRA^{R} matrix (LifeSciences, Plainsboro, NJ, USA). An INTEGRA^{R} patch 20 × 15 cm in size (Figure 3B) was placed over the gap of 7 × 5 cm with exposed bone, thereafter affixed and covered with an antimicrobial dressing (Figure 3C). No vacuum therapy was performed. The wound was inspected five days after the placement of the INTEGRA^{R} matrix. On the 18th postoperative day, the outer silicon layer was removed and neodermis was formed, which measured 22 × 18 cm (Figure 3D). Ultrathin split-thickness skin 1:1.5 meshed autograft, harvested from the left calf, was applied over the neodermis. The wound was completely healed with stable coverage. Postoperative course was uneventful, and a six-month follow-up revealed resistant tissues on both sides, right and left. With no contracture, with normal skin pliability and normal range of movement, but also with superior quality of scars on the side treated by the INTEGRA^{R} matrix. We were more satisfied with the side covered by the INTEGRA^{R} matrix.

We confirm that we have read the journal's position on issues involving ethical publication and affirm that this work is consistent with those guidelines. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration

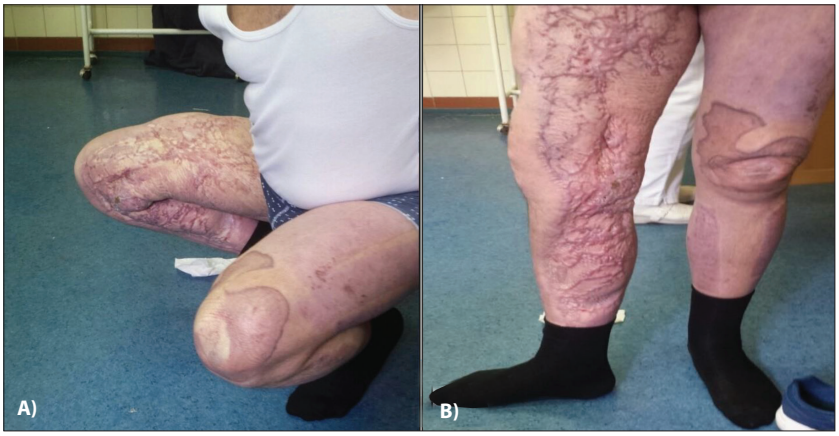

Figure 4. Ambulatory follow-up after six months; full range of motion of the left knee joint without contracture as well as satisfactory aesthetic result (A); comparing the quality of scars, skin texture, and pliability on the right and the left side show better results on the left side (B)

and its later amendments or comparable ethical standards. Written consent to publish all shown material was obtained from the patient.

DISCUSSION

Burn wound closure within the first five days is optimal, but this is often difficult to achieve in polytrauma patients, with concurrent traumatic injuries. Although debridement of full-thickness burns and autologous grafting remains the gold standard for treatment of third-degree burns, there are some challenging occasions, when we have to use skin substitutes with biological properties. Whenever the available skin donor sites are limited or the overall patient condition does not permit the coverage of excised burn wounds with autologous skin, there may still be a clinical demand for human allograft skin as a temporary biologic dressing [6]. As there is no skin bank in our country, we used skin allografts from living human donors.

Regarding the depth, reconstruction of soft tissue defects, resulting from the debridement of full-thickness burns, may extend deeper, to exposed bone, with denuded periosteum. These defects are not amenable to skin grafting; thus, a flap is needed, which is already standard care for lower-extremity injuries with exposed bone [7–10]. However, this option may be unavailable because of not only inadequate adjacent tissues but also due to poor overall condition or is technically difficult to perform [11]. Lee et al. [10] presented that INTEGRA^R matrix provides stable, long-term coverage for lower-limb burn injuries with exposed structures, with better aesthetic results compared to prolonged granulation followed by skin grafting or bulky tissue flaps, and allows the coverage of vital structures when flaps are unavailable or not a good option. Guerra et al. [11] have also noted an extraordinary capacity for INTEGRA^R to bridge avascular gaps in the wound bed in very deep burns to the extremities over small areas of bone and tendon. Interestingly, we highlighted the area with exposed bone without periosteum on the left knee, which was covered by INTEGRA^R, because our patient was not a candidate for flap reconstruction. Accordingly, as in our case, INTEGRA^R may be indicated to cover deep wounds, especially in weakened patients who are not eligible for flap rearrangement [12]. Regarding the anatomical site, the knee is largely a subcutaneous joint, which has to be promptly and properly covered with well-vascularized tissue. Various options have been used in the reconstruction of these defects: local muscle flaps, fasciocutaneous flaps, and free flaps [13, 14]. Products such as INTEGRA^R achieved optimal results, which "challenge the current gold-standard treatment" for lower-extremity defects with the anti-scarring effects – thus, they promote better aesthetic results with less resultant scarring [15].

INTEGRA^R artificial skin was developed by the cooperative work of the Massachusetts General Hospital and the Institute of Technology in the 1970s. Additionally, the first described use of INTEGRA^R was by Yannas and Burke [16]. INTEGRA^R dermal regeneration template is a dual-layer regeneration template composed of cross-linked bovine collagen and glycosaminoglycan from shark cartilage coated with an outer thin temporary epidermal substitute layer of a polysiloxane polymer (silicone) [16]. Its architecture provides ideal physicochemical conditions, leading to dead space elimination, control of bacterial invasion, prevention of water loss, while simultaneously ensuring cell migration with vascular growth, which are important for neodermis formation. Since its introduction in 1981, it has been successfully used for burn injuries [17]. Infection remains the most common complication of INTEGRA^R use, underlining the need for careful wound bed excision and meticulous hemostasis [18]. Despite, the main reason for its limited use in clinical practice is certainly its high cost [19]. However, since the introduction, several studies have been published from all over the world, proving its ability to vascularize over small areas of exposed bone and tendon [10, 11, 20, 21]. Ben-Nakhi and Eltayeb [21] concluded that INTEGRA^R was easy to use, safe, and effective when used over exposed underlying structures in the wound bed, including bones, tendons, and joints.

As in our case, many reports suggest that long-term results using INTEGRA^R lead to skin elasticity with no evidence of hypertrophic scar formation or clinical contracture [22]. There is some evidence which described combined application of negative pressure wound therapy (NPWT) and dermal substitutes [23]. NPWT is the application of a negative pressure across a wound to improve tissue repair and regeneration. The first commercially available NPWT device marketed in the United States was the Vacuum-Assisted Closure (VAC). Therefore, VAC uses

negative pressure to prepare the wound for spontaneous healing or for other reconstructive options.

Early surgical debridement was of great importance for patient survival. Unlike standard methods of repair, we used alternative methods such as skin substitutes as well. Further, our case showed that the INTEGRA^{R} matrix prevents functional disability and, furthermore, it leads to optimal aesthetic results.

Conflict of interest: None declared.

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Изазови у хирургији дубоких опекотина

Биљана Ћертић[1], Анђела Димкић-Миленковић[1], Милан Јовановић[1,2]

[1]Универзитетски клинички центар Србије, Клиника за опекотине, пластичну и реконструктивну хирургију, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија

САЖЕТАК

Увод Опекотине пуне дебљине коже представљају значајан изазов у погледу хируршког лечења, посебно када истовремено постоје и повреде других органа. Традиционални третман дубоких опекотина укључује рану ексцизију или дебридман некротичног ткива, после чега следи пресађивање коже или реконструкција режњем. Постоје бројни изазови, као што су лоше опште стање пацијента, повреде других органа, упитна вијабилност подлоге ране, ограничене давајуће регије, када морамо узети у разматрање супституенте коже. ИНТЕГРА је ацелуларни дермални супституент која ствара нативни дермис.

Циљ овог рада је био да поделимо наше искуство лечења супституентима коже код политрауматизованог пацијента са опекотинама.

Приказ болесника Приказујемо 46-годишњег мушкарца са тешким контактним опекотинским ранама заједно са вишеструким преломима ребара и пршљенова, као и контузијом плућа са локализованим билатералним хемотораксом. Пацијент је задобио опекотине трећег степена доњих екстремитета, које су захватале скротални и глутеални део и обухватиле 15% укупне површине тела. Подвргнут је раној ексцизији некротичног ткива десне ноге са накнадном аутотрансплантацијом коже, међутим, због делимичног лизирања аутотрансплантата били смо принуђени да урадимо алотрансплантацију праћену каснијом аутотрансплантацијом због ограничених места давајуће регије и лошег општег стања пацијента. Сукцесивни дебридмани и парцијална остектомија довели су до дефекта левог колена са експонираном пателом. Дакле, у циљу реконструкције последичног дефекта и превенције контрактуре зглоба, дефект је финално покривен ИНТЕГРОМ.

Закључак Наше искуство је показало да ИНТЕГРА спречава функционалну онеспособљеност и, поред тога, доводи до оптималних естетских резултата.

Кључне речи: ИНТЕГРА; опекотине пуне дебљине коже; експонирана кост; кожни трансплантати; режњеви; реконструкција

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Giant left lobe hemangioma of the liver misdiagnosed for splenomegaly

Borislav Tošković[1,2], Vladimir Milosavljević[1], Matija Buzejić[3], Nataša Stanisavljević[2,4], Darko Zdravković[2,5]

[1]Bežanijska Kosa University Hospital Medical Center, Department for HPB Surgery, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Department for Surgery with Anesthesiology, Belgrade, Serbia;
[3]University Clinical Center of Serbia, Clinic for Endocrine Surgery, Belgrade, Serbia;
[4]Bežanijska Kosa University Hospital Medical Center, Department for Hematology, Belgrade, Serbia;
[5]Bežanijska Kosa University Hospital Medical Center, Department for Oncology Surgery, Belgrade, Serbia

SUMMARY
Introduction Most patients with liver hemangiomas are unrecognized, when symptoms occur it is usually due to the size of the hemangioma. Hemangioma of the liver are benign tumors which affects women more often. Surgical indications for liver resection remain unclear.
Case outline We present a patient with a giant hemangioma of the left liver lobe that was misdiagnosed in a primary care unit. The patient underwent resection of the left liver lobe and fully recovered after several days.
Conclusion Symptoms, size, and risk of rupture should be considered when decision for surgery is made. Linear stapler can be useful especially when left and middle hepatic vein have common trunk.
Keywords: liver; hemangioma; surgery; liver resection

INTRODUCTION

Liver hemangioma is one of the most common benign lesions of the liver, which can affect 20–30% of the general population. It is more diagnosed in women than in men with ratio up to 5:1 [1]. Hemangiomas are usually diagnosed incidentally on computer tomography (CT), magnetic resonance (MRI), or ultrasound (US) of the abdomen. They usually grow silently but it can be manifested as abdominal pain if it grows larger than 10 cm. Hemangiomas larger than 4 cm are diagnosed as giant liver hemangiomas based on literature [2, 3]. Pathogenesis is not clear, but it is a congenital vascular malformation or a hamartoma [4]. At histopathology exam, it is usually revealed as a mesenchymal lesion consisting of blood-filled vascular cavities of different sizes, surrounded by a simple layer of flat endothelial cells, supported by a fibrous connective tissue.

The aim of our work is to present a rare giant hemangioma of the liver initially interpreted as splenomegaly. We also present the operative technique and a review of the current literature.

CASE REPORT

A 45-year-old female patient was admitted to the hospital at the Hematology Department for further examination as massive splenomegaly was verified on an abdominal US. After admission to our hospital, we performed a CT scan of the abdomen, which showed a discrepancy in relation to the US finding of the abdomen. Namely, a liver tumor in the left lobe was verified, after which an MRI with retrograde cholangiopancreatography was performed and the CT findings were confirmed – more precisely, a giant tumor of the left lobe of the liver was verified, occupying the left hypochondriac and left lumbar quadrants of the abdomen. The tumor around 22 cm in diameter dislocated the spleen towards the pelvis was (Figure 1). The patient reported discomfort in the abdomen and decreased appetite. Laboratory values were between the reference ranges, including alpha fetoprotein, carcinoembryonic antigen, and cancer antigen 19-9. Based on the performed diagnostics and laboratory parameters, the conclusion was that it has been a giant hemangioma of the liver. An indication for operative treatment was established. Firstly, embolization of the left branch of the hepatic artery was attempted in order to possibly reduce the volume of the tumor, but, for technical reasons, the procedure was not performed successfully. Since the tumor was bigger than 20 cm in diameter, surgical team opted for "J" laparotomy. After incision, when we approached the abdomen, a giant-left-lobe liver was presented that occupied the left side of the abdomen. The next step was extrahepatic dissection, after identification of the left side of the hepatoduodenal ligament. The left hepatic artery was taped, followed by the left portal vein being taped, and dissected

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Correspondence to:
Vladimir MILOSAVLJEVIĆ
Bežanijska Kosa University Hospital Medical Center
Department for HPB Surgery
Dr Žorža Matea bb
11080 Belgrade
Serbia
milosavljevicvladimir10@gmail.com

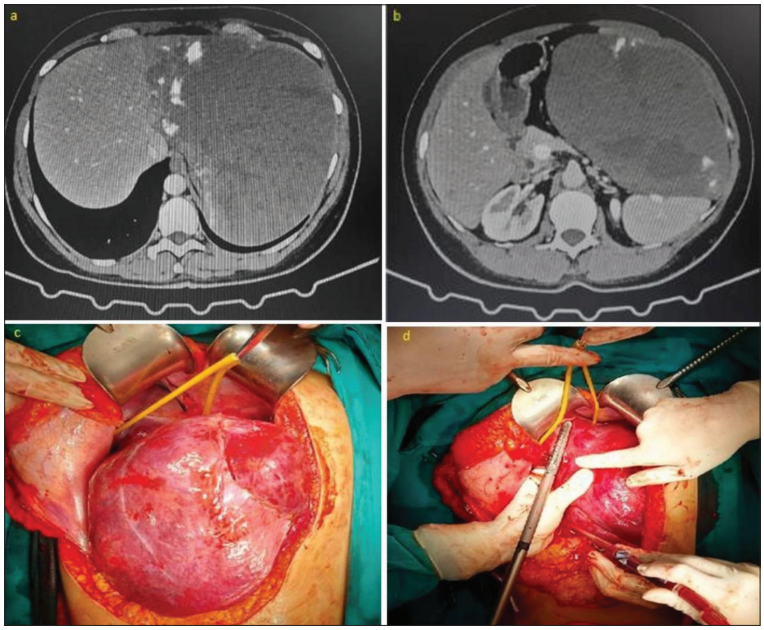

Figure 1. a, b – Magnetic resonance showing enlarged left lobe of the liver due to hemangioma; c, d – intraoperative finding and placed linear stapler

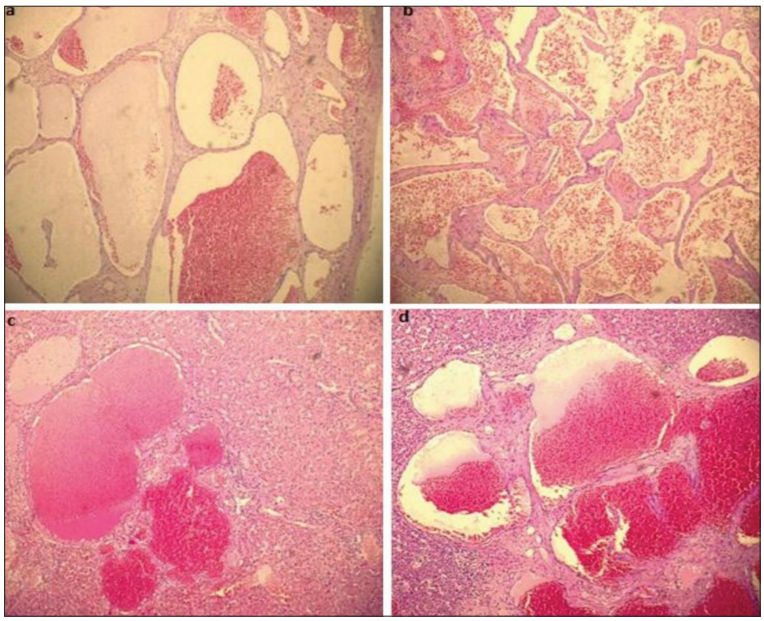

Figure 2. a, b, c, d – Pathological images, showing proliferation of blood vessels with thinned walls covered by endothelium without atypia (H&E, 5 ×)

as far as the root of the right portal vein. The left hepatic duct was identified just above the left portal vein. The falciform, right and left coronary, and left triangular ligaments were incised to mobilize the left lateral section. The inferior vena cava ligament, which fixes the caudate lobe to the cava from behind, was ligated and divided. After these steps, parenchymal dissection was begun on the inferior surface and was continued along the middle hepatic vein with linear stapler (Figure 1). The left and the middle hepatic vain had a common trunk, so the stapler was very useful. The tumor weight was 3200 g after being pulled out from the abdomen. The postoperative period was uneventful, and the patient was discharged from hospital on the fifth postoperative day. A histopathological finding revealed that the tumor consisted mainly of a large number of abnormally dilated blood sinuses, there were no signs of endothelial atypia (Figure 2).

DISCUSSION

Liver hemangioma is the most common hepatic tumor, and it more often affects women than men. The reason for this sex-related occurrence inequality may be that some hemangiomas express estrogen receptors – those hemangiomas tend to grow during pregnancy and oral contraceptive drug use [1, 5]. Previously, hepatic hemangiomas larger than 5 cm in diameter were considered giant hepatic hemangiomas. However, data from the active literature indicate that hemangiomas with a diameter greater than 10 cm should be considered giant, which is more in line with the characteristics of the tumor and the requirements for diagnosis and treatment [6]. Extremely giant hepatic hemangiomas (> 10 cm) are rare and generally asymptomatic. When the symptomatology is present, it is mostly related to the compressive effect on the surrounding organovascular structures, resulting in a feeling of discomfort in the abdomen, pain, nausea, etc. [7, 8].

The patient we are presenting was of medium ostemuscular structure, and her chief problem was the size of the liver tumor. Namely, she had a feeling of heaviness and bloating in her stomach, inability to lie on her left side, frequent nausea.

The diagnostic methods for hepatic hemangioma include US, CT, MRI, scintigraphy, and positron-emission tomography combined with CT, angiography [9]. At US it usually presents as a hyperechogenic lesion with posterior acoustic enhancement. In CT, the density of the lesion is the same as the vessels. In MRI, it presents as homogenous and hyperintense on T2-weighted images, hypointense on T1-weighted images [10, 11].

In our case, we applied a CT protocol for hemangioma [12], and also performed MRI of the abdomen with retrograde cholangiopancreatography in order to fully assess the relationship of the tumor with the biliary ducts and surrounding organovascular structures.

In addition to abdominal discomfort and the size of the lesion, indications for surgery include spontaneous or traumatic rupture, rapidly enlarging lesions, Kasabach–Merritt syndrome, and unclear diagnosis (suspect of malignancy) [8, 13]. Treatments may be radiofrequency ablation, monoclonal antibody therapy, radiation therapy, trans-arterial embolization, interferon therapy, liver transplantation, and surgical procedures (enucleation or resection) [14, 15].

General consideration between surgical approaches is that enucleation is performed in a shorter operative time, but the capsule of hemangioma rupture bleeding is hard to get under control; in contrast, during resection when left hepatic vein is occluded, and pringle maneuver is made along with decreased central venous pressure, operative time can be shortened, and bleeding can be less. Also, intraoperative venous bleeding can be reduced by lowering central venous pressure and portal vein pressure by reducing collateral vessel filling, which helps to reduce intraoperative venous bleeding [16].

Since the hemangioma in our patient occupied almost the entire left lobe of the liver, along with the fact that the arterial embolization was not successfully performed, we decided on surgical treatment, i.e. resection of the liver using the stapler technique.

Hemangioma of the liver is a benign disease, which, however, can cause certain problems that impair the quality of life of patients. Operative treatment or resection of the liver should be carefully considered when the tumor grows, if it is larger than 10 cm in diameter and if patients have pronounced symptoms. Liver transplantation should be considered in fewer cases if a giant hemangioma is present in both lobes (due to localization). Liver resection is a safe and effective surgical procedure. Also, the application of a linear stapler can be useful especially when left and middle hepatic vein have a common trunk.

Ethical standards: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest: None declared.

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Велики хемангиом левог лобуса јетре иницијално протумачен као спленомегалија

Борислав Тошковић[1,2], Владимир Милосављевић[1], Матија Бузејић[3], Наташа Станисављевић[2,4], Дарко Здравковић[2,5]

[1]Универзитетска болница „Бежанијска коса", Одељење хепатобилијарне хирургије, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Катедра за хирургију и анестезиологију, Београд, Србија;
[3]Универзитетски клинички центар Србије, Клиника за ендокрину хирургију, Београд, Србија;
[4]Универзитетска болница „Бежанијска коса", Одељење хематологије, Београд, Србија;
[5]Универзитетска болница „Бежанијска коса", Одељење онколошке хирургије, Београд, Србија

САЖЕТАК

Увод Већина хемангиома јетре је непрепозната код болесника, а када се симптоми појаве, то је углавном због величине промене. Хемангиоми јетре су бенигни тумори, који су чешћи у женској популацији. Консензус око одлуке о операцији и ресекцији јетре не постоји, изузев када су у питању руптура хематома и крварење.

Приказ болесника Болесница је примљена у болницу под сумњом на спленомегалију, а даљим радиолошким испитивањем доказан је велики тумор левог режња јетре. С обзиром на величину промене, учињена је ресекција левог режња јетре са хемангиомом. Болесница је у добром општем стању отпуштена из болнице четвртог постоперативног дана.

Закључак Величина, симптоми и ризик од руптуре хемангиома треба да буду главни критеријуми када се доноси одлука о оперативном лечењу хемангиома јетре. Коришћење стаплера може бити корисно приликом ресекције, посебно када лева и средња хепатична вена имају заједнички трункус.

Кључне речи: јетра; хемангиом; хирургија; ресекција јетре

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Association of bilateral inferior vena cava with azygos and hemiazygos continuation and aortic coarctation in a child

Staša Krasić[1], Vesna Topić[2], Vladislav Vukomanović[1,3]

[1]Dr. Vukan Čupić Mother and Child Health Care Institute of Serbia, Department of Cardiology, Belgrade, Serbia;
[2]Dr. Vukan Čupić Mother and Child Health Care Institute of Serbia, Department of Radiology, Belgrade, Serbia;
[3]University of Belgrade, Faculty of Medicine, Belgrade, Serbia

SUMMARY

Introduction Developmental variations of the inferior vena cava (IVC) rarely cause symptoms, and they are usually detected during routine examinations performed for other reasons. The prevalence in the general population is between 0.07% and 8.7%. Various anomalies of the IVC can be seen depending on abnormal regression or abnormal persistence of embryonic veins. They are usually associated with more complex intracardiac and atrioventricular septal defects, partial anomalous pulmonary venous connection, and pulmonary atresia.

Case outline We presented an 18-month-old patient with double IVC, IVC interruption, azygos, and hemiazygos continuation associated with aortic coarctation. The vein malformation was discovered during percutaneous balloon angioplasty of the aortic re-coarctation and confirmed by using cross-sectional imaging modalities. Our patient had no symptoms of IVC malformation.

Conclusion In clinical practice, double IVC should be suspected in patients with recurrent pulmonary emboli. Another important point in practice is the identification of those anomalies to avoid potential complications of retroperitoneal surgery and cannulation during cardiac surgery.

Keywords: double inferior vena cava; interruption of inferior vena cava; hemiazygos continuation; azygos continuation

INTRODUCTION

Developmental variations of inferior vena cava (IVC) are described with the incidence in the general population between 0.07% and 8.7%, and they are primarily asymptomatic [1]. They are usually associated with more complex intracardiac and spleen lesions (polysplenia/asplenia syndrome), but in some patients, they were detected during routine examinations performed for other reasons [2–6]. The most frequent congenital abnormalities include circumaortic left renal vein in 1.5-8.7% of patients, 2.1% patients had retroaortic left renal vein, 0.2–3% had double IVC, 0.6% had azygos or hemiazygos continuation of IVC, and isolated left-sided IVC was found in 0.2–0.5% [7]. These anomalies reflect the complicated and multisegmental character of the IVC development during embryogenesis [3–6].

We presented a two-year-old child with aortic coarctation, IVC duplication, intrahepatic agenesis and azygos and hemiazygos continuation.

CASE REPORT

A newborn was admitted to our Institute due to a prenatal diagnosis of the hypoplastic left chamber and aortic arch. At the admission, the echocardiographic finding pointed out a poorly developed left ventricle, bicuspid aortic valve (BAV), aortic coarctation and pulmonary hypertension. Minor atrial and perimembranous ventricular septal defects and intrahepatic IVC agenesis were described. At six days old, aortic coarctation surgery was performed. During the follow-up period, on echocardiography examinations, re-coarctation was observed. At 18 months old, percutaneous balloon angioplasty was performed. During the heart catheterization, IVC malformation with intrahepatic IVC agenesis was observed (Figure 1).

MDCT examination of the abdomen was performed for better evaluation of venous anomalies. The double IVC was formed by the confluence of the ipsilateral right and left common iliac veins, and the duplication was seen on both sides of the aorta (Figure 2A). The right renal vein joins into the right IVC and the left into the left IVC. The IVCs had two communications: at the level of common iliac veins and renal veins with retroaortic course (Figure 2B). The intrahepatic agenesis of the IVC was described, with azygos and hemiazygos continuation of the right and left IVC. The hemiazygos vein joins the azygos vein at the usual place (Figure 2C), and the azygos vein drains into the superior vena cava (SVC). Hepatic veins drain into the right atrium.

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Correspondence to:
Vladislav A. VUKOMANOVIĆ
Dr. Vukan Čupić Mother and Child Health Care Institute of Serbia
Radoja Dakića 6–8
11070 Belgrade
Serbia
vvukomanovicdr@gmail.com

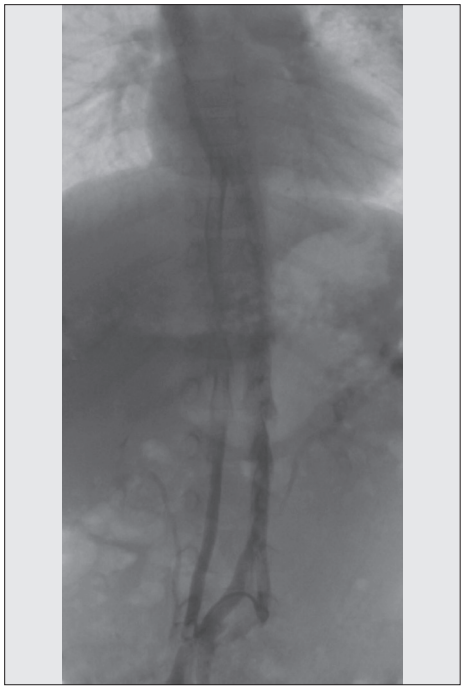

Figure 1. Coronary plane – double inferior cava vein with intrahepatic agenesis, hemiazygos and azygos continuation

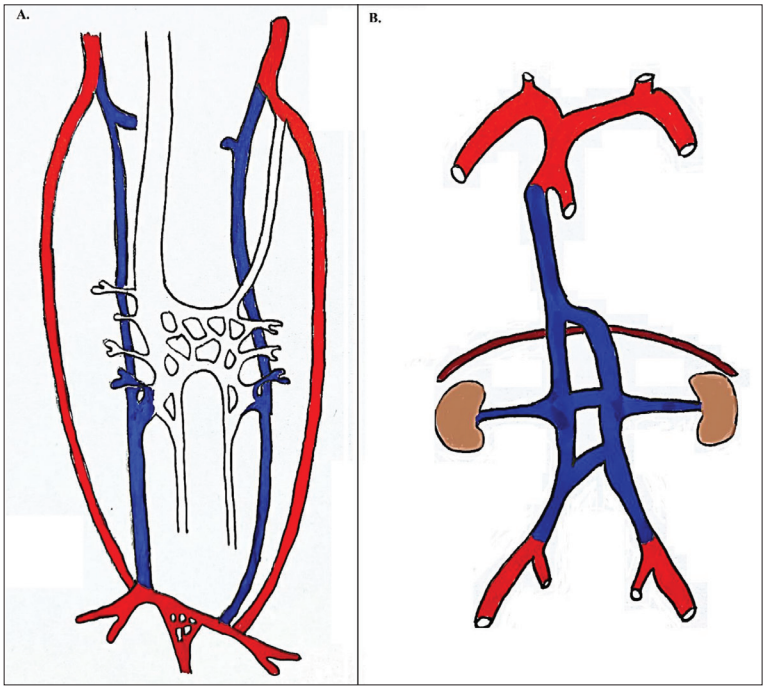

Figure 3. A – schematic diagram showing early formation of the posterior cardinal veins (red), subcardinal veins (white), and supracardinal veins (blue); B – interruption of the hepatic segment of double inferior vena cava (right and left) with hemiazygos and azygos continuation; the left inferior vena cava reaches the azygos vein through the hemiazygos vein; the subcardinal part is missing; supracardinal veins (blue) persist bilaterally

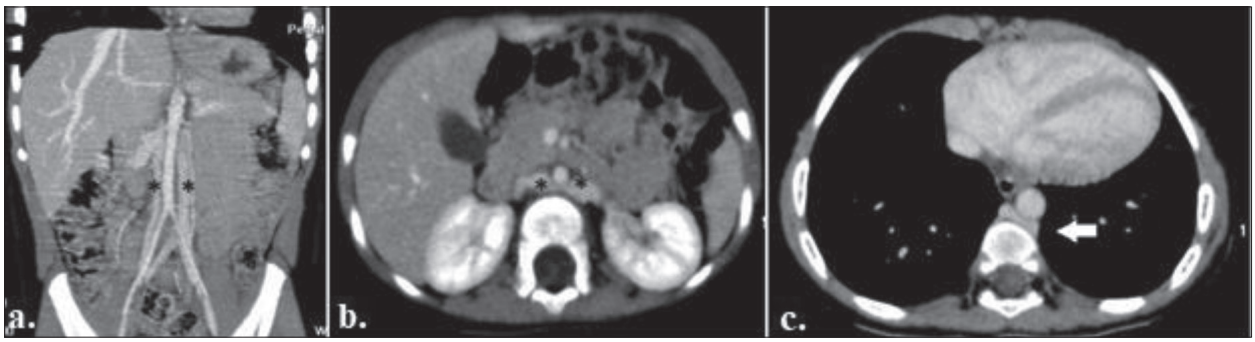

Figure 2. a – Inferior vena cava duplication, with interiliac communication from the right common iliac vein (type 2c); b – retroaortic communication at the level of renal veins; c – hemiazygos vein drain into azygos vein at the usual place; asterisk – inferior vena cava; arrow – hemiazygos – azygos drain place

The local ethics committee approved the manuscript. Written informed consent was obtained from the patient's parents to publish any potentially identifiable images or data in this article.

DISCUSSION

The retroperitoneal venous system undergoes development between the 4th and 8th gestational week. The IVC development is a complicated embryological process including the growth, regression, midline anastomoses, and replacement of three pairs of embryological veins (posterior cardinal, subcardinal, and supra cardinal veins) and the vitelline veins [1–5]. The infrahepatic IVC develops from a set of three paired parallel cardinal veins, while the cranial segment of the right vitelline vein emanates suprahepatic IVC. The retrohepatic segment is derived from an anastomosis between the cranial segment of the right subcardinal vein and the right vitelline vein (Figure 3 A) [2–6]. Varied IVC anomalies can be seen pivoting on abnormal regression or persistence of embryonic veins [3].

The prevalence of bilateral IVCs is 0.2–3%, resulting from the persistence of both right and left supracardinal veins (failure of regression) [1–6]. The most typical setup implies two distinct IVCs originate from each iliac vein, as in our case. However, in most cases, the left IVC terminates at the level of the left renal vein, crossing over to join the right IVC [2–7]. Wolfhard et al. [7] presented a patient with bilateral IVC with azygos continuation and the supradiaphragmatic join of hemiazygos and azygos vein. This patient was like ours, but our patient had two connections between IVCs and associated congenital anomalies (double IVC type 2c [8]).

Interruption of IVC is distinguished by the lack of suprarenal IVC, with a preponderance of 0.6–2.1%. It is the consequence of ineffectual anastomosis of the right subcardinal and hepatic vein, and on the other side atrophy of the right subcardinal vein (maldevelopment). Consequently, owing to agenesis of the hepatic tract of the IVC, the blood circulating in the caudal segments of the vessel reaches the azygos system, partially emanating from the right supra cardinal vein [1–5, 8, 9]. Both azygos and hemiazygos continuation occur, but azygos continuation is more frequent [4]. Although our patient had double IVC with azygos and hemiazygos continuation, the supracardinal system is entirely persistent (Figure 3B). Interruption of IVC is characterized by the absence of suprarenal IVC, with a prevalence of 0.6–2.1%. It is caused by failure of the right subcardinal-hepatic anastomosis and atrophy of the right subcardinal vein (loss of development). Consequently, owing to agenesis of the hepatic tract of the IVC, the blood circulating in the caudal segments of the vessel reaches the azygos system, partially derived from the right supra cardinal vein [1–5, 8, 9]. Both azygos and hemiazygos continuation occur, but azygos continuation is much more common [4]. Although our patient had double IVC with azygos and hemiazygos continuation, the supracardinal system is entirely persistent (Figure 3B).

Hemiazygos and azygos continuation of the IVC with SVC interruption has been commonly related to *situs inversus*, asplenia, polysplenia syndromes, atrioventricular septal defects, partial anomalous pulmonary venous connection, and pulmonary atresia [1, 3, 4, 5, 8]. Our patient had BAV, aortic coarctation and a poorly developed left ventricle; however, vein malformation was detected during balloon angioplasty. A case with BAV, aortic coarctation and uninterrupted left-sided IVC was described in the literature, but this patient had unilateral IVC [10]. Karadeniz et al. [11] presented an adult patient with aortic coarctation, double SVC, a left-hand side IVC with hemiazygos vein continuation, and a right retroaortic renal vein with polysplenia/heterotaxy syndrome.

The prevalence of thromboembolic complications in patients with double IVC is unexplored, but the literature data showed that those patients had an increased incidence of thrombosis. In patients with recurrent embolic disease, IVC filter placement may be impacted by anomalies of the IVC and may require different techniques for each anomaly [1, 2, 7, 10]. Patients with azygos continuation might also develop sick sinus syndrome [1].

Congenital anomalies such as double IVC are rare and require no intervention. This case demonstrates an 18-month-old patient with double IVC, IVC interruption, azygos, and hemiazygos continuation associated with aortic coarctation. The vein malformation was discovered in the catheterization room and confirmed using cross-sectional imaging modalities. Our patient had no symptoms of IVC malformation. In clinical practice, double IVC should be suspected in patients with recurrent pulmonary emboli. Another important point in clinical practice is the identification of those anomalies to avoid potential complications of retroperitoneal surgery and cannulation during cardiac surgery.

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Повезаност билатералне доње шупље вене са азигос и хемиазигос континуацијом и коарктацијом аорте код детета

Сташа Красић[1], Весна Топић[2], Владислав Вукомановић[1,3]

[1]Институт за здравствену заштиту мајке и детета Србије „Др Вукан Чупић", Служба кардиологије, Београд Србија;
[2]Институт за здравствену заштиту мајке и детета Србије „Др Вукан Чупић", Служба за радиологију, Београд Србија;
[3]Универзитет у Београду, Медицински факултет, Београд, Србија

САЖЕТАК

Увод Развојне аномалије доње шупље вене (ДШВ) ретко изазивају симптоме, а откривају се углавном током рутинских прегледа из других разлога. Преваленција у општој популацији је између 0,07% и 8,7%. Аномалије ДШВ могу бити последица абнормалне регресије или перзистирања ембриоланих вена. Обично су удружени са сложенијим урођеним срчаним манама, као што је атриовентрикуларни септални дефект, парцијалним аномалним уливом плућних вена и атрезијом плућне артерије.

Приказ болесника Приказали смо 18-месечног болесника са двоструком ДШВ, прекидом ДШВ, азигос и хемиазигос континуацијом са коарктацијом аорте. Малформација ДШВ је откривена током перкутане балонске ангиопластике рекоарктације аорте, а потврђена је компјутеризованом томографијом. Болесник није имао симптоме малформације ДШВ.

Закључак У клиничкој пракси треба сумњати на дуплу ДШВ код болесника са рекурентном плућном емболијом. Такође, важна је идентификација ових аномалија како би се избегле могуће компликације ретроперитонеалне хирургије и канулације током кардиохируршких процедура.

Кључне речи: дупла доња шупља вена; прекид доње шупље вене; азигос и хемиазигос континуација

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CASE REPORT / ПРИКАЗ БОЛЕСНИКА

Photocoagulation for retinal hemangioblastoma in Von Hippel–Lindau disease

Jelena Vasilijević[1,2], Jelica Pantelić[1,2], Jelena Mirković[1], Leila Al Barri[3], Igor Kovačević[1,2]

[1]University Clinical Centre of Serbia, Clinic for Eye Diseases, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[3]Victor Babes University of Medicine and Pharmacology, Department of Ophthalmology Timisoara, Romania

SUMMARY

Introduction Von Hippel–Lindau disease is a hereditary, autosomal dominant, tumor syndrome with a predisposition to developing various benign and malignant tumors. Retinal hemangioblastoma is often the presenting manifestation. We report a case of Von-Hippel–Lindau disease in a 13-year-old girl with bilateral eye involvement.

Case outline The patient was referred to the Eye Clinic, University Clinical Center of Serbia, with a diagnosis of Coats disease. Clinical examination revealed that best corrected visual acuity was 20/20 on her right eye, while her left eye showed counting fingers at 20 cm distance. Dilated fundoscopy of the right eye revealed multiple tortuous feeding vessels leading to orange-reddish, sharply demarcated multiple lesions on the far periphery of the upper retina, corresponding to retinal hemangioblastoma. the left eye showed edematous optic nerve head, tortuous retinal vessels, exudates, and retinal detachment including macula. Considering that the patient had multiple bilateral retinal hemangioblastomas and that her father had pathohistologically proven brain hemangioblastoma and numerous visceral tumors, Von Hippel–Lindau disease was assumed. Focal argon laser photocoagulation was performed in the region of all visible vascular tumors and feeding vessels in the right eye. The patient's visual acuity remained unchanged five months after the disease detection.

Conclusion The importance of education about dominant inheritance pattern of Von Hippel–Lindau disease cannot be overemphasized. Role of an ophthalmologist is critical in early diagnosis of both retinal hemangioblastoma and Von Hippel–Lindau disease.

Keywords: Von Hippel–Lindau disease; retinal hemangioblastoma; photocoagulation

INTRODUCTION

Von Hippel–Lindau disease (VHL) is a hereditary, autosomal dominant tumor syndrome. Patients are predisposed to develop various benign and malignant tumors, especially retinal hemangioblastomas (RH), central nervous system (CNS) hemangioblastomas, and renal tumors; however, other neoplasms can also occur, including adrenal gland, pancreatic, inner ear, epididymal, and endolymphatic sac tumors. The disease arises from a VHL tumor suppressor gene mutation located on the third chromosome. It usually presents in early adulthood. The penetrance is over 90% until the seventh decade [1, 2]. Incidence varies internationally from around one in 36,000 to one in 91,000 [1].

RH is often the presenting manifestation of the disease [2]. Clinically, the tumor appears as red, orange, or pink, well-demarcated oval lesions, with tortuous and dilated feeding vessels. The peripheral retina and the optic nerve head (ONH) can be affected. Exudation is often seen around the lesion and in the macular region [1, 3]. Bilateral involvement and multiple RH are usual [3, 4].

Diagnosis and screening of VHL demand a multidisciplinary approach in which an ophthalmologist plays an important role. Timely diagnosis is essential since the disease is both vision- and life-threatening. Genetic testing and clinical assessment are recommended in patients with known family history even when asymptomatic, as well as in all patients with hemangioblastoma. Screening consists of regular physical evaluation, ophthalmic and audiological examination, brain and abdominal magnetic resonance imaging (MRI), all of these beginning from early childhood [1, 2].

We report a case of VHL in a 13-year-old girl with bilateral eye involvement.

CASE REPORT

A 13-year-old girl was urgently referred from a regional eye center to the Eye Clinic, University Clinical Center of Serbia, with retinal detachment in the left eye and diagnosis of Coats disease. Medical, ocular, and family history were taken from the girl's mother. She reported that girl was healthy, had no known medical conditions, had a full-term delivery, and complete immunization was performed. The history of ocular diseases and trauma was also negative. While taking a detailed family history, we found

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Correspondence to:
Jelena B. VASILIJEVIĆ
University Clinical Centre of Serbia
Clinic for Eye Diseases
Pasterova 2
Belgrade 11000
Serbia
bkjelena@gmail.com

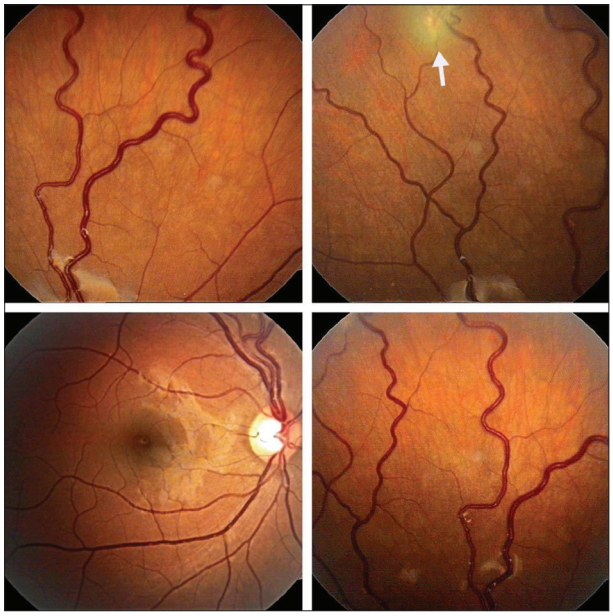

Figure 1. Color photos of the right eye on admission where a single retinal hemangioblastoma is clearly identifiable in extreme superior periphery (arrow)

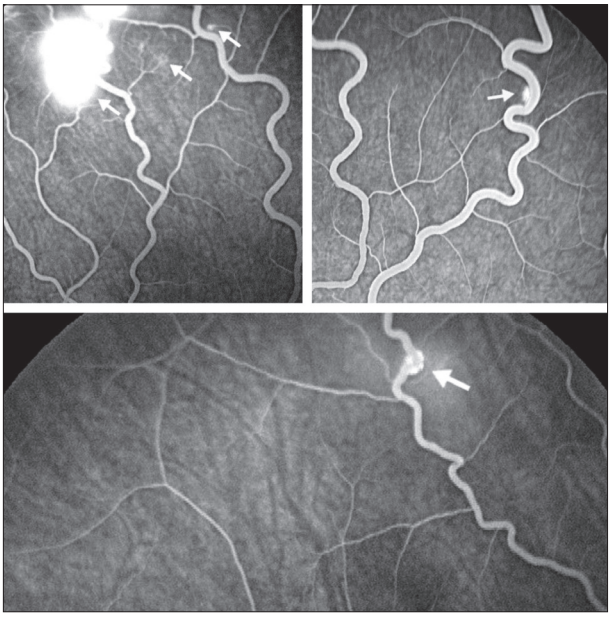

Figure 3. Fluorescein angiography of the right eye on admission, showing areas of leakage in the superior periphery, which corresponds to retinal hemangioblastomas (arrows)

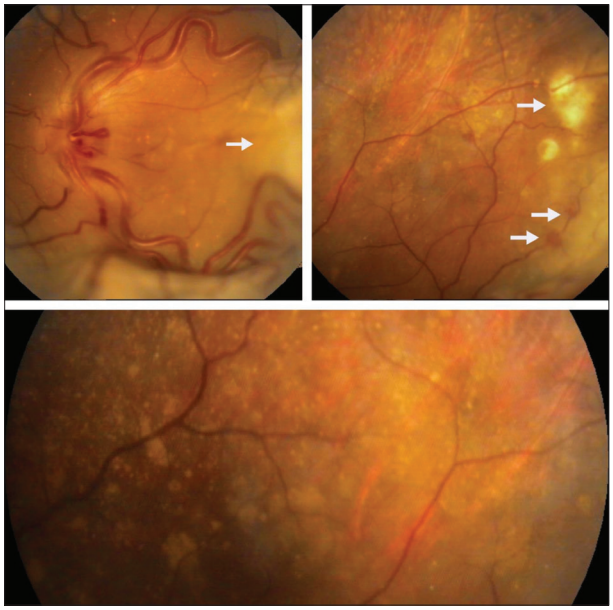

Figure 2. Color photos of the left eye on admission showing retinal detachment with underlying retinal hemangiomas (arrows)

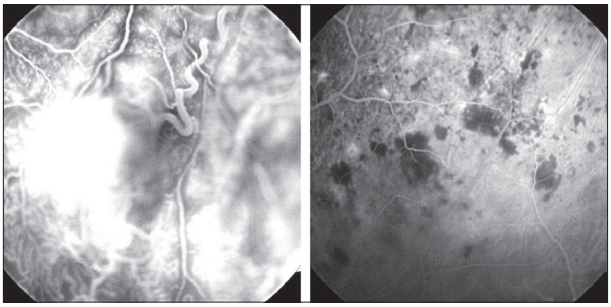

Figure 4. Fluorescein angiography of the left eye of typical peripheral retinal hemangioblastomas showing early hyperfluorescence and marked late-phase leakage while subretinal exudation was seen as blocked hyperfluorescence

out that the girl's father had died two months prior from kidney disease. Additionally, 17 years prior, he had a brain tumor surgery.

A clinical examination revealed that best corrected visual acuity measured on the Snellen chart was 20/20 on her right eye, while her left eye showed counting fingers at 20 cm distance. Intraocular pressures were within normal limits. The left pupil was slightly wider and the light reflex was slower. Both anterior segments and eye motility were normal. Dilated indirect fundoscopy of the right eye revealed multiple tortuous feeding vessels leading to orange-reddish, sharply demarcated multiple lesions on the far periphery of the upper retina, corresponding to RH. The macular region and ONH of the right eye were unaffected (Figure 1). The left eye showed edematous ONH, tortuous retinal vessels, exudates, and retinal detachment including macula (Figure 2). Optical coherence tomography and optical coherence tomography angiography could not be performed on the left eye due to massive exudation and retinal detachment, while both were normal on the right eye. The patient was admitted to the hospital for further evaluation and treatment. On admission, b-scan ultrasonography and fluorescein angiography were conducted. Ultrasonography of the right eye was normal, while, on the left eye, it showed a hemorrhagic retinal detachment of the lower retinal parts, including the macular region, with a large intraretinal cyst. Fluorescein angiography of the right eye showed tortuous feeding vessels with early and marked late phase leakage from the clinically visible tumors (Figure 3). Left fluorescein angiography corresponded with clinically described retinal detachment with diffuse leakage from retinal vessels of the upper, attached retina and underlying RH (Figure 4). Prompt focal argon laser photocoagulation (LPC) was performed in the region

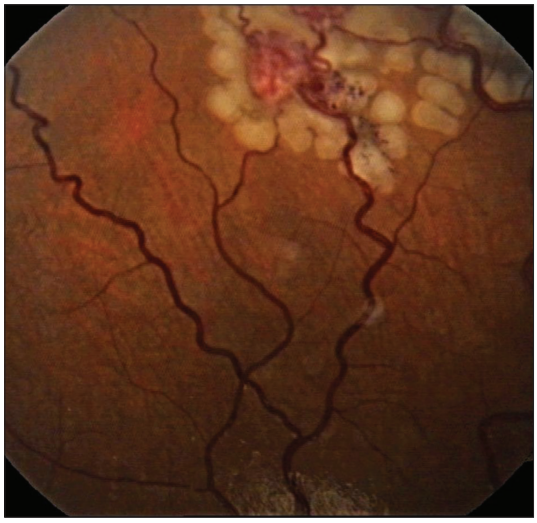

Figure 5. Color photo of the right fundus after the first focal laser photocoagulation treatment

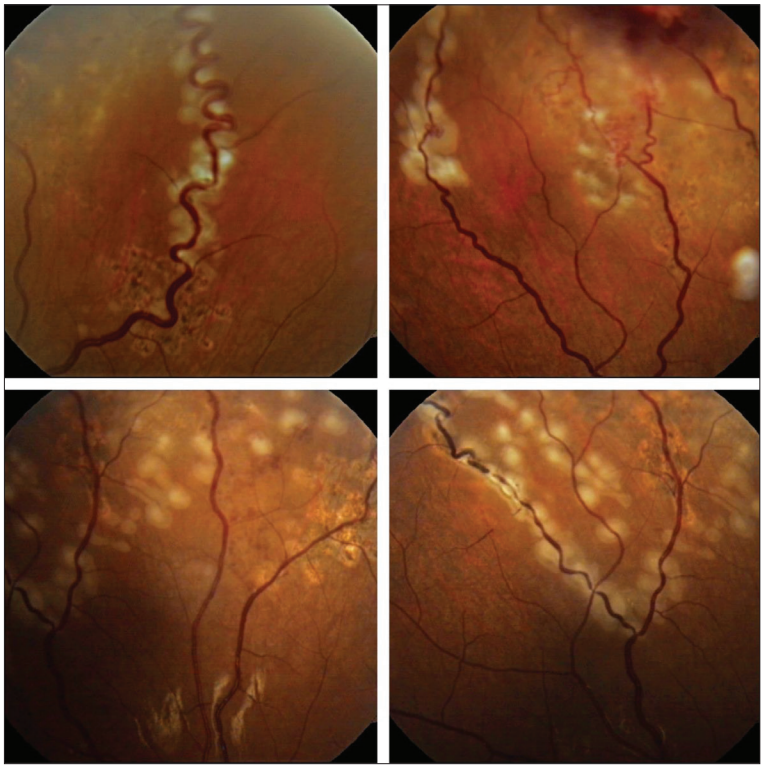

Figure 6. Color photos of the right eye after additional focal laser photocoagulation treatments a month (top row) and two months (bottom row) after discharge

of all visible vascular tumors in the right eye in order to prevent visual loss on the only functional eye (Figure 5). Considering that the patient had multiple bilateral RH and that her father had a brain lesion and kidney disease, VHL was assumed. During hospitalization, brain MRI, pediatric, and nephrological physical exams were obtained, which were all normal.

On the first follow-up, 10 days after discharge, partial vitreous hemorrhage and bleeding over the tumor were observed. LPC spots were partially pigmented. The visual acuity on the right eye was 20/20. The left eye findings were unchanged. A thorough inspection of the father's medical documentation revealed that he had pathohistologically proven brain hemangioblastoma. Furthermore, he was diagnosed with multiple kidney and pancreatic tumors and suspected to have VHL, while additional diagnostics could not be performed due to his poor general health, at that time. On the second and third follow-ups (a month and two months after discharge, respectively), partial tumor regression was observed; still, additional LPC treatment was applied to the multiple tumor lesions and feeding vessels of the right eye to ensure the prevention of further complications (Figure 6). Regular monthly follow-ups have been conducted and the patient's visual acuity on the right eye remained stable five months after the disease detection.

This case report was approved by the institutional ethics committee, and written consent was obtained from the patient for the publication of this case report and any accompanying images.

DISCUSSION

RH can be either sporadic or, more often, a part of the VHL. A Danish retrospective national study from 2018 reported genetically confirmed VHL in 84% of their patients with RH [5]. These findings highlight the importance of thinking about VHL in all patients with RH and distinguishing cases with sporadic disease of those associated with VHL, since malignant visceral neoplasms could be present in the latter form.

VHL disease is typically confirmed through genetic testing. Individuals with first-degree relatives suffering from VHL should also undergo genetic testing in order to detect those with the risk of tumor evolution and provide them with an appropriate surveillance. If genetic testing is unavailable, the diagnosis can be established based on clinical criteria of at least one VHL manifestation and a first-degree relative with confirmed disease or at least two VHL tumors affecting different organs, of which at least one has to be hemangioblastoma [1, 2]. According to these standards, we can conclude that our patient's father could have been clinically diagnosed with VHL, as well as our patient based on RH findings and positive family history. The patient's mother was unaware of the disease's hereditary nature and the need for surveillance of all descendants. When family history is present, screening should ideally consist of genetic testing and/or evaluation of organ systems diseases connected to VHL. The screening protocol for RH starts from the first year and includes a dilated indirect fundoscopy once a year [1, 2, 6, 7].

RH represents one of the most common tumors found in VHL, occasionally diagnosed in childhood, as presented in our case [1, 2]. The average age of presentation is 25 years, being the lowest compared to other VHL-related tumors. The earliest reported onset was in the first year of life [6]. Additionally, patients diagnosed with VHL have

an earlier occurrence of RH compared to sporadic cases. Consequently, RH is the first sign of VHL in up to 77% of patients, which emphasizes the important role of an ophthalmologist in disease detection [2–6]. A cohort study that consisted of 335 subjects, examined the epidemiology of RH in VHL and found no correlation to age, sex, and laterality. The same study noticed a higher prevalence of bilateral RH (57.9%), a predilection for peripheral retina compared to juxta-papillary RH (84.7%), and an average tumor number of 2.5 ± 1.8 per eye [8]. Our patient had bilateral disease and multiple tumors with typical appearance, affecting the peripheral retina, which encompasses characteristic clinical presentation of VHL. Still, she was referred to our institution with suspicion of Coats disease. When retinal exudation is present, Coats disease and retinal macroaneurysm can be a differential diagnosis; however, our patient did not have visible retinal exudation on her right eye. Other conditions that may resemble RH include retinal vasoproliferative tumors, microvascular abnormalities, congenital retinal arteriovenous malformations and papillitis, juxta-papillar choroiditis or choroidal neovascularization in case of juxta-papillary RH [7]. For that reason, examination of the dilated fundus is important to identify any existing changes, especially in the far periphery, where changes can easily be overlooked.

RH is benign in terms of its biological features. It can, however, lead to visual impairing complications which correlate with the tumor size. Exudation from the lesion, vitreous hemorrhage and traction of the nearby retina are the commonest [8, 9]. It is a slow-growing tumor that sometimes allows observation with close follow-up instead of prompt treatment. Even so, treatment is required in most cases. Specific guidelines for the treatment of RH have not been published yet, therefore decision on the most appropriate treatment method is based on clinical experience and literature data. LPC, with the aim to destruct the tumor or the feeding vessels, is the mainstay of treatment, except for RH near the macula or ONH [10, 11]. Krivosic et al. [9] treated 100 eyes with LPC alone and reported success with inactivating RH in 97% of subjects while maintaining baseline visual acuity during the next four and a half years. The success rate was absolute in cases of small RH (less than 1 disc diameter) [9]. LPC carries a risk of bleeding or subretinal exudation and cannot be used when complications, such as exudative or tractional retinal detachment occur [12]. Therefore, early intervention, when the tumor is still small, can significantly affect the outcome. Other treatment modalities include cryotherapy, photodynamic therapy, transpupillary thermotherapy, radiotherapy, intravitreal anti-vascular endothelial growth factor therapy, and pars plana vitrectomy [10, 11, 12]. A recent review report suggests that the tumor size followed by tumor location and concomitant complications are the main factors in choosing a therapeutic option. They propose that small peripheral tumors (less than 1.5 mm) without tumor-related complications should be treated with LPC. Medium-sized lesions (1.5–4.5 mm) could be managed with either cryotherapy, transpupillary thermotherapy, or radiotherapy. For tumors larger than 4.5 mm and those with vitreoretinal complications, vitreorectomy is preferable [11]. Juxta-papillary RH are a specific therapeutic challenge. Photodynamic therapy in these cases can be beneficial [9, 12]. Combined therapies are also reported [9, 13]. Our patient's right eye was successfully treated with LPC and the visual acuity was unchanged during a five-month-long follow-up. However, she still has profound visual impairment in her left eye, which could have been prevented if ophthalmological examinations had been performed as recommended, from the first year of life.

The importance of education about the disease-dominant inheritance pattern and adequate screening of patients with VHL and their close relatives cannot be overemphasized. The role of an ophthalmologist is critical in early diagnosis of both RH and VHL since the disease can be blinding or even lethal.

Conflict of interest: None declared.

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Ласерска фотокоагулација за ретинални хемангиобластом у Фон Хипел–Линдауовој болести

Јелена Василијевић[1,2], Јелица Пантелић[1,2], Јелена Мирковић[1], Лејла ал Бари[3], Игор Ковачевић[1,2]

[1]Универзитетски клинички центар Србије, Клиника за очне болести, Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија;
[3]Универзитет медицине и фармакологије „Виктор Бабеш", Одсек за офталмологију, Темишвар, Румунија

САЖЕТАК

Увод Фон Хипел–Линдауова болест је аутозомно доминантно наследни туморски синдром са предиспозицијом за развој различитих бенигних и малигних тумора. Ретинални хемангиобластом је често прва манифестација болести. Приказујемо случај Фон Хипел–Линдауове болести код 13-годишње девојчице са захватањем оба ока.

Приказ болесника Болесница је упућена на Клинику за очне болести Универзитетског клиничког центра Србије са дијагнозом Коатсове болести. Клинички преглед показао је најбоље кориговану видну оштрину 20/20 на десном оку, а на левом оку бројање прстију на удаљености од 20 центиметара. Преглед десног ока у мидријази показао је бројне извијугане исхрањујуће крвне судове који су водили до бројних наранџасто-црвенкастих, оштро ограничених лезија на крајњој периферији горње ретине, који су одговарали ретиналним хемангиобластомима. Лево око је показало оток главе очног нерва, извијугане крвне судове, ексудате и аблацију ретине која је захватала макулу. Узимајући у обзир да је болесница имала бројне обостране ретиналне хемангиобластоме и да је њен отац имао патохистолошки доказан хемангиобластом мозга као и разне висцералне туморе, постављена је сумња на Фон Хипел–Линдауову болест. Фокална фотокоагулација са аргонским ласером је учињена у регији свих видљивих васкуларних тумора и исхрањујућих крвних судова на десном оку. Видна оштрина је остала непромењена пет месеци после откривања болести.

Закључак Значај едукације о доминантном типу наслеђивања Фон Хипел–Линдауове болести не може бити пренаглашен. Улога офталмолога је кључна у раној дијагнози како ретиналног хемагниобластома, тако и Фон Хипел–Линдауове болести.

Кључне речи: Фон Хипел–Линдауова болест; ретинални хемангиобластом; фотокоагулација

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REVIEW OF LITERATURE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Celiac disease – a comprehensive review

Nedeljko Radlović[1], Zoran Leković[2,3], Marija Mladenović[4], Biljana Vuletić[5,6], Jelena Radlović[7], Siniša Dučić[2,3], Dejan Nikolić[2,3]

[1]Serbian Medical Society, Academy of Medical Sciences, Belgrade, Serbia;
[2]University Children's Hospital, Belgrade, Serbia;
[3]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[4]Singidunum University, Faculty of Health, Legal and Business Studies, Valjevo, Serbia;
[5]University Clinical Center of Kragujevac, Pediatric Clinic, Kragujevac, Serbia;
[6]University of Kragujevac, Faculty of Medical Sciences, Kragujevac, Serbia;
[7]Railways of Serbia Institute for Health Care of Workers, Belgrade, Serbia

SUMMARY
Celiac disease is a multisystemic autoimmune disease induced by gluten in wheat, rye, and barley. It is characterized by polygenic predisposition, prevailing prevalence in members of the white population (1%), especially in close relatives (5–15%), very heterogeneous expression and frequent association with other autoimmune diseases (3–10%), as well as selective deficiency of IgA and Down, Turner, and Williams syndromes. The basis of the disease and the key finding in its diagnostics is gluten-sensitive enteropathy, i.e., non-specific inflammation of the small intestinal mucosa which resolves by gluten-free diet. In addition to enteropathy, whether symptomatic or asymptomatic, the disease is also characterized by various extraintestinal manifestations, and even very serious complications. Therapy is based on a lifelong gluten-free diet, so that the disorder, if diagnosed in time and treated consistently, has an excellent prognosis.
Keywords: celiac disease; pathogenesis; clinical forms; diagnostics

INTRODUCTION

Celiac disease (CD) is a systemic autoimmune disease induced by gluten of wheat, rye, and barley in genetically predisposed individuals [1, 2, 3]. It occurs in all population groups, and most often in members of the white race (1:100) [3–6]. As to other autoimmune diseases, it is more frequent in people of the female versus male sex (1.5:1 to 2:1) [7, 8, 9]. It is particularly common in first- and second-degree relatives (5–15%) [10]. With a slightly lower frequency (3–10%) it occurs in patients with other autoimmune diseases, selective IgA deficiency and Down, Turner, and Williams syndromes [1, 3, 6, 11–16].

The main feature of the disease and the basis of its diagnosis is non-specific inflammation of the small intestinal mucosa that resolves on a gluten-free diet [1, 3, 6, 17, 18]. In addition to damage of the small intestinal mucosa, which can be symptomatic or asymptomatic, the disease is also characterized by numerous extraintestinal manifestations and in cases diagnosed too late or treated inconsistently, permanent consequences and sometimes very serious complications [3, 4, 9, 19–25].

PATHOGENESIS

The pathogenetic basis of CD is polygenic predisposition and exposure to gluten containing cereals [2, 3, 26]. Gluten is a complex water insoluble protein that comprises about 75–80% of the total proteins of wheat, rye, and barley flour [27]. It is characterized by a high content of glutamine and proline-rich polypeptide residues resistant to efficient gastrointestinal proteolysis, which, after passing into the submucosa of the small intestine, lead to inadequate immune reactions in genetically predisposed individuals [2, 26, 28]. In addition to gluten, gastrointestinal infections, alteration of the intestinal microbiota, some medications, and other factors play an important role in the occurrence of the disease, which explains its incomplete prevalence in monozygotic twins (83–86%) [3, 10, 11, 17, 29, 30]. Longer breastfeeding and continuation of breastfeeding after gluten introduction delay the onset of CD [31]. Evidence of the prevalent role of polygenic inheritance in the occurrence of the disease, its highly variable frequency in different populations, as well as its high presence in identical twins and first-degree relatives (~10%) [10, 11, 13, 32]. The human leukocyte antigens (HLA) class II genes *DQ2* and *DQ8* (6p21.32), which are present in 98–99% of patients, play a central role in the hereditary predisposition to the disease [6, 10, 26, 33]. HLA *DQ2* molecules are registered in 85–95% of patients, and HLA *DQ8* in 5–15% [34, 35, 36]. In addition, HLA *DQ2*/*DQ2* homozygotes have a particularly high risk of developing CD, as well as its earlier onset and more severe form of manifestation, including more frequent occurrence of complications, such as "celiac crisis," refractory type of the disease

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Correspondence to:
Nedeljko RADLOVIĆ
Serbian Medical Society
Džordža Vašingtona 19
110000 Belgrade
Serbia
n.radlovic@beotel.net

and enteropathy-associated T-cell lymphoma [3, 9, 29, 37]. However, apart from HLA *DQ2* or *DQ8* genes and exposure to gluten, the presence of one or more of the approximately 40 non-HLA genes (e.g., *TAGAP, IL18R1, RGS21, PLEK,* and *CCR9*) that have been verified so far is indispensable for the appearance disease [3, 11, 13, 26].

The importance of *DQ2* and *DQ8* glycoproteins present on antigen-presenting cells (dendritic cells and macrophages) in the pathogenesis of CD is reflected in their ability to activate intestinal CD4+ T-cells after binding with deaminated gluten polypeptide hydrolysates [2, 3, 11]. The deamidation of gluten hydrolysates, which increases the affinity of their binding to HLA *DQ2* and *DQ8* molecules, is performed by tissue transglutaminase (tTG). Proinflammatory cytokines released by activated CD4+ T-cells parallel activate intraepithelial cytotoxic CD8+ T-cells which lead to enterocyte apoptosis and infiltrative or infiltrative-destructive inflammation of the small intestine mucosa and the differentiation of B lymphocytes into plasma cells and the production of antibodies against gluten peptides and autoantibodies to endomysium (EMA) and tTG [10, 26].

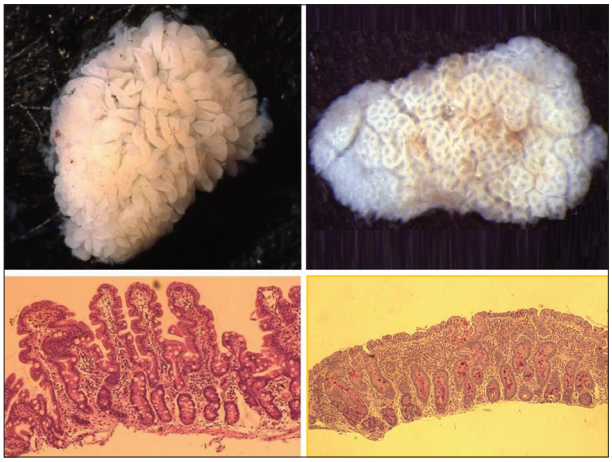

Figure 1. Stereomicroscopic and pathohistological appearance of the normal small intestinal mucosa (left) and in the state of the most severe damage (Marsh IIIc) (right); the right stereomicroscopic image shows a lack of intestinal villi with crypt openings, and pathohistologically, apart from the absence of villi, crypt hyperplasia with pronounced lympho-plasmocytic infiltration of the lamina propria (original recordings were made by the authors)

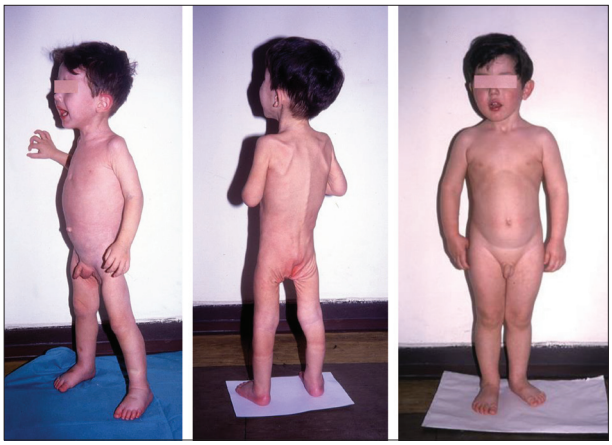

Figure 2. A two-year-old boy with the classic form of celiac disease; on the left and in the middle in the diagnostic phase, and on the right after 12 months of a gluten-free diet and the first two weeks of lactose restriction; the images in the diagnostic phase, along with the typical clinical aspect, show hypoproteinemic edema on the lower extremities, conspicuous loss of muscle and fat tissue in the gluteal region ("tobacco bag phenomenon") and perianal erythema as a consequence of secondary lactose intolerance (original recordings were made by the authors with parental permission and consent)

ENTEROPATHY

Enteropathy (morphological damage to the mucosa of the small intestine) is most pronounced in the duodenum and the proximal part of the jejunum and progressively decreases towards the ileum [38]. In some cases, however, evident mucosal lesions may be present only in the duodenal bulb [1, 6, 17]. According to the modified Marsh criteria, inflammation of the small intestine mucosa is classified into three basic forms: infiltrative (I), infiltrative-hyperplastic (II), and destructive (III) [39]. In the first form of mucosal damage, there is an increased number of intraepithelial lymphocytes with γ/δ receptor properties, as well as lympho-plasmocytic infiltration of the stroma, while the height of the intestinal villi and the depth of the crypts remain preserved. In the second type of damage, in addition to more pronounced infiltrative changes, there is hyperplasia of the crypts, while in the third, with additional accentuated infiltration and hyperplasia of the crypts, shortening and/or loss of villi occurs. According to the degree of mucosal damage, destructive enteropathy is further classified into partial (IIIa), subtotal (IIIb), and total (IIIc) (Figure 1). Apart from that, a fourth form of damage is also possible, which is characterized by complete atrophy of the villi, but without crypt hyperplasia and typical signs of mucosal inflammation.

CLINICAL FORMS OF THE DISEASE

Observed from the aspect of manifestation, there are two basic clinical forms of CD: symptomatic and asymptomatic (subclinical) [1, 2, 17]. Within the framework of the symptomatic disease, forms with classic and non-classical clinical presentation are distinguished [1, 2, 17]. The classical form of the disease is characterized by chronic diarrhea followed by malabsorption and secondary malnutrition, while the clinical picture of the non-classical disease is dominated by extraintestinal manifestations [1, 2, 17, 22, 23]. The classic form of the disease is most often seen in infants and young children, and the non-classic in later ages and in adults [11, 17, 23]. In the symptomatic form of the disease, along with evident enteropathy, autoantibodies to tissue transglutaminase (AtTG) and EMA, as well as the HLA *DQ2* and/or *DQ8* genotype, are almost regularly registered [1, 2, 12, 33, 35]. However, despite the presence of all these indicators, in most cases, CD remains unmanifest for a long time, and this form of the disease is called subclinical ("silent CD") [1, 2]. In addition, potential CD has an asymptomatic character, which differs from the

previous one in the normal appearance of the small intestine mucosa [1, 2, 33]. In a significant number of patients with potential CD, enteropathy is also registered later [1].

In youngest children aged 9–36 months, CD almost regularly occurs in the clinical classic form [9, 17]. It is characterized by a relatively short period after the introduction of gluten into the menu, a gradual onset and a progressive course manifested by chronic diarrhea, anorexia, occasional vomiting, abdominal distension, apathy and irritability [17]. As a consequence of insufficient intake and malabsorption of nutrients, global malnutrition occurs, accompanied by sideropenic anemia, loss of fat tissue and reduction of bone and muscle mass (Figure 2) [40, 41]. In the most severe cases, secondary lactose intolerance, isolated hypertransaminasemia ("celiac hepatitis"), and sometimes the appearance of hypoproteinemic edema is registered [20, 41]. Within the first 6–9 months after birth, the disease usually has a rapid and severe clinical course. In rare cases, the so-called "celiac crisis" characterized by total gastrointestinal insufficiency followed by severe dehydration, metabolic acidosis, meteorism, drastic weight loss and hypoproteinemic edema [9].

The onset and course of the disease in preschool children is predominantly non-classical (atypical) [17]. Compared to earlier age, gastrointestinal disturbances are less often present or absent. Recurrent abdominal pain and constipation, sometimes diarrhea, and often sideropenic anemia, poor appetite, malnutrition, stagnation in longitudinal growth and a change in the child's personality are encountered.

In the symptomatology of the disease in later childhood and adolescence mono or oligosymptomatic extraintestinal manifestations dominate [17]. In addition to the manifestations seen in preschool age, there are others, such as maturation delay, enamel hypoplasia, recurrent aphthous stomatitis, chronic malaise, dermatitis herpetiformis, osteopenia, arthralgia, myalgia, cerebellar ataxia, polyneuropathy, epilepsy and others [3, 6, 17, 22, 42].

The most frequent symptoms of CD in adulthood are: anemia, chronic fatigue, weight loss, recurrent abdominal pain, bloating, flatulence, constipation, mouth ulcer, headaches, depression, and osteopenia or osteoporosis [19, 43, 44]. In addition, women have an increased risk for infertility, miscarriage, and early menopause [19].

In about 1–1.5% of total cases of CD, mostly in adulthood, refractory CD (RCD) occurs [3, 11]. This type of disease is characterized by malabsorption, weight loss, as well as persistent villous atrophy and after a year a strict gluten-free diet [2, 6]. RCD may be categorized in two subtypes – type 1, where the phenotype of the intraepithelial lymphocyte population is normal (CD3+CD8+); type 2, where it is abnormal [3, 11]. RCD, particularly type 2, is associated with serious complications, such as ulcerative jejunitis and enteropathy-associated T-cell lymphoma [3, 19, 45].

Although the classic form of the disease is the most often described and best studied entity, today it is known that it represents only the "tip of the celiac iceberg" and that the largest number of patients, both children and adults, are those with a non-classical and asymptomatic form of the disease [17].

ASSOCIATION WITH OTHER DISEASES

In addition to the high frequency among close relatives of the patient, especially those of the first degree, CD is characterized by a high association (3–10%) with other autoimmune diseases, such as diabetes mellitus type I, autoimmune thyroiditis, Addison's disease, rheumatoid arthritis, juvenile idiopathic arthritis, Sjögren's syndrome, systemic lupus erythematosus, autoimmune liver diseases, IgA nephropathy, myasthenia gravis, psoriasis, dilated cardiomyopathy, autoimmune pericarditis, and others [1, 6, 11–15]. Approximately the same prevalence of the disease occurs in selective IgA deficiency, as well as in Down, Turner, and Williams syndrome [1, 3, 13]. Therefore, serological screening for CD is indicated in first degree relatives, as well as in the mentioned autoimmune and chromosomal patients [3, 6, 16, 17, 33, 46].

DIAGNOSIS

The diagnosis of CD, except in cases explained in the following text, is based on an enterobiopsy with pathohistological examination of the small intestine mucosa [1, 3, 6, 17, 18]. Biopsies are obtained from the duodenum via an upper gastrointestinal endoscopy, whereby one or two from the bulb and ≥ 4 from the distal duodenum [1, 3, 17]. Such a diagnostic approach is necessary because the histologic changes may be patchy in distribution and confined to the duodenal bulb. In order for the conditions for pathohistological analysis to be adequate, the correct orientation of the biopsies is required [47, 48].

The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), as part of the recommendations published in 2012, unlike earlier ones, considers that enterobiopsy is not necessary in patients with symptoms and/or signs consistent with CD, and in addition, they have an IgA titer antibodies to tissue transglutaminase (AtTG-IgA) ≥ 10 times above the upper reference value, positive anti-endomysial antibodies of the same class (EMA-IgA) and "celiac HLA" (*DQ2* and/or *DQ8*) [1]. The clinical recovery of the patient and the disappearance of AtTG are part of the confirmation of the disease, i.e., the justification for the introduction of a gluten-free diet without a previous enterobiopsy. This attitude in the diagnosis of CD is based not only on the high sensitivity and specificity of AtTG-IgA as a serological marker of the disease (> 95%), but also on the highly significant correlation of their titer with the degree of damage to the mucosa of the small intestine, as well as the almost inevitable correlation (> 98%) presence of HLA *DQ2* and/or *DQ8*. An additional difference compared to the previous position is that even in children under two years with an exact diagnosis of CD, provocation of gluten tolerance with pathohistological analysis of the small intestine mucosa is not required. However, in patients in

whom a gluten-free diet was introduced without a previous enterobiopsy, as well as in cases where the morphological damage of the mucosa was not typical or the samples were inadequate for a reliable interpretation, the final confirmation or exclusion of CD is based on the enterobiopsy and pathohistological findings during the provocation of gluten tolerance. Because it can endanger the quality of permanent teeth, this procedure is not recommended before the age of six, and because of the side effects on the child's growth and development during puberty.

ESPGHAN, as part of the additional modification of the criteria for the diagnosis of CD, published in January 2020, does not consider enterobiopsy with pathohistological analysis of small intestine mucosa samples necessary even in asymptomatic patients with a serum level of AtTG-IgA class ≥ 10 times above the upper reference level values and positive EMA-IgA in a second serum sample [33]. Also, bearing in mind the almost absolute association of CD and HLA *DQ2* and/or *DQ8* in these patients, as well as in those whose diagnosis was established by enterobiopsy, testing in this sense is not necessary. However, in all other cases, the diagnosis of CD requires strict adherence to the 2012 criteria. It is additionally recommended that, as part of the initial serological screening for CD, with prior verification of normal serum IgA for age, AtTG-IgA should be used, and not EMA and antibodies to deamidated gliadin peptide (AtDGP). However, if it is a suspected patient with IgA deficiency, tests based on IgG class antibodies (AtDGP, EMA or AtTG) should be used for this purpose. If there is a discrepancy between the level of AtTG-IgA and the pathohistological findings, it is necessary to re-evaluate the appearance of the biopsy or consult another pathologist. Patients with elevated serum levels of AtTG-IgA and EMA-IgA in whom normal or minimally damaged mucosa of the small intestine (Marsh 0/I) was registered, require strict monitoring.

Except in the mentioned exceptions, serological tests for CD have no diagnostic value [1, 6]. Hence, they are primarily used in the detection of asymptomatic and non-classical forms of the disease and in the assessment of the consistency of the elimination diet in patients in whom it has been established [1]. When interpreting serological screening, it should be borne in mind that it can be positive even without the characteristic damage of the small intestinal mucosa, which is seen in other autoimmune diseases and other pathological conditions [1]. Contrary to this, due to the immunological immaturity of children under two years, AtTG may be negative despite evident enteropathy [6, 17]. For this reason, when screening children under two years of age for CD, the IgA tTG test should be combined with AtDGP (IgA and IgG) [6, 17]. In the last ten years in the detection of CD, as well as gluten-free diet monitoring, the tTG-based commercial rapid whole blood test is widely used, which is less invasive, more practical and cheaper than the serological test [49].

The update recommendations of the American College of Gastroenterology (ACG) on the diagnosis of CD in children and adults published this year do not differ from the current ESPGHAN recommendations [6, 33]. The American Gastroenterological Association also agrees with the ESPGHAN and ACG guidelines in the diagnosis of CD in children, with the fact that in adults, for purposes of differential diagnosis, esophagogastroduodenoscopy with duodenal biopsy can also be performed [18]. However, the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition in its 2016 criteria for the diagnosis of CD includes endoscopic enterobiopsy as a mandatory [17]. This attitude is based on the fact that without an endoscopic diagnostic approach, comorbid conditions with CD, such as peptic and eosinophilic esophagitis or *Helicobacter pylori* gastritis, can be overlooked. The European Society for the Study of CD also advocates the necessity of enterobiopsy in the diagnosis of CD in adults [3].

THERAPY

Patients with CD should adhere to a gluten free diet for life [1, 3, 6, 17, 18]. Most of them with a symptomatic form of the disease, especially the classical one, during the initial phase of treatment require the correction of micronutrient deficits, primarily iron and folate, and sometimes temporary restriction of lactose [6, 41]. In patients with "celiac crisis," in addition to the correction of hydroelectrolyte and acid-base imbalance and removal of edema, semi-elemental and/or parenteral nutrition is applied, and sometimes short-term glucocorticoid therapy [3, 9]. RCD therapy also requires parenteral nutrition and immunosuppressive therapy containing steroids, azathioprine, 6-mercaptopurine, and methotrexate, whereas RCD2 therapy is based on additional medications, including cyclosporine and chemotherapy such as cladribine and fludarabine associated with anti-CD52 monoclonal antibodies (alemtuzumab) [3, 11, 50]. RCD1 usually responds to a gluten-free diet, nutritional support, and immunosuppressive medications, while the therapeutic response in RCD2 is incomplete and, accordingly, prognosis is often poor [6, 45].

PROGNOSIS

The prognosis of timely recognized and adequately treated CD is excellent [11, 39]. Delayed recognition of the disease or non-compliance with the elimination diet, however, can lead to serious consequences, including serious complications, both during the growth and development, and those that manifest in adulthood, such as enteropathy-associated T-cell lymphoma, small bowel adenocarcinoma, osteoporosis, miscarriages, infertility, and others [3, 9, 11, 19, 21, 23, 25].

CONCLUSION

CD represents a polygenically determined autoimmune disorder induced by gluten of wheat, barley, and rye. It primarily occurs in Caucasians, and particularly often in close relatives of the diseased, as well as in patients with other autoimmune diseases, selective IgA deficit, and Down, Turner,

and Williams syndrome. The basis of the disease and the key finding in its diagnostics are formed by the non-specific inflammation of the small intestinal mucosa that resolves by gluten-free diet. Beside enteropathy, either symptomatic or asymptomatic, the disease is also characterized by various extraintestinal manifestations, and in neglected cases, serious complications. The therapy is based on lifelong gluten-free diet, and this disorder, if timely diagnosed and adequately treated, has an outstanding prognosis.

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The authors declare that the article was written according to ethical standards of the Serbian Archives of Medicine as well as ethical standards of medical facilities for each author involved.

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Целијачна болест – свеобухватан преглед

Недељко Радловић[1], Зоран Лековић[2,3], Марија Младеновић[4], Биљана Вулетић[5,6], Јелена Радловић[7], Синиша Дучић[2], Дејан Николић[2,3]

[1]Српско лекарско друштво, Академија медицинских наука, Београд, Србија;
[2]Универзитетска дечја клиника, Београд, Србија;
[3]Универзитет у Београду, Медицински факултет, Београд, Србија;
[4]Универзитет „Сингидунум", Факултет здравствених, правних и пословних студија, Ваљево, Србија;
[5]Универзитетски клинички центар Крагујевац, Клиника за педијатрију, Крагујевац, Србија;
[6]Универзитет у Крагујевцу, Факултет медицинских наука, Крагујевац, Србија;
[7]Завод за здравствену заштиту радника „Железнице Србије", Београд, Србија

САЖЕТАК

Целијачна болест је мултисистемско аутоимунско обољење индуковано глутеном пшенице, ражи и јечма. Карактерише је полигенска предиспозиција, преовлађујућа преваленца код припадника беле популације (1%), посебно код блиских сродника (5–15%), веома хетерогена експресија и честа удруженост са другим аутоимунским болестима (3–10%), као и селективним дефицитом ИгА и Дауновим, Тарнеровим и Вилијамсовим синдромом. Основу болести и кључни налаз у њеној дијагностици чини ентеропатија осетљива на глутен, тј. неспецифично запаљење слузокоже танког црева које се повлачи на дијети без глутена. Поред ентеропатије, било симптоматске или асимптоматске, болест карактеришу и различите екстраинтестиналне манифестације, па и веома озбиљне компликације. Терапија се заснива на доживотној дијети без глутена, тако да поремећај, ако се благовремено дијагностикује и доследно лечи, има одличну прогнозу.

Кључне речи: целијачна болест; патогенеза; клинички облици; дијагностика

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REVIEW OF LITERATURE / ПРЕГЛЕД ЛИТЕРАТУРЕ

Optical coherent tomography with angiography in glaucoma

Ivan Marjanović[1,2], Vesna Marić[2,1], Marija Božić[1,2]

[1]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[2]University Clinical Center of Serbia, University Eye Hospital, Belgrade, Serbia

SUMMARY
Optical coherence tomography with angiography (OCT-A) is a non-invasive imaging technique that enables three-dimensional visualization of perfusion through the vascular network of the retina and choroid. The possibilities of OCT-A for glaucomatologists will expand in the near future. The possibility of detecting and monitoring glaucoma with this technology will also be expanded. All of these systems will undoubtedly offer software updates, making it easier for examiners to use the device itself, and thus monitor the disease. OCT-A represents the future in the diagnosis of retinal diseases and glaucoma.
Keywords: glaucoma; optical coherence tomography; angiography

INTRODUCTION

Optical coherence tomography with angiography (OCT-A) is a non-invasive imaging technique that enables three-dimensional visualization of perfusion through the vascular network of the retina and choroid.

Unlike standard OCT, OCT-A analyzes not only the intensity of reflected light, but also the temporal changes in reflection caused by the movement of particles, such as erythrocytes circulating through blood vessels [1]. These changes are detected in OCT signals as repeated freezing of OCT images of each part of the retina, thus forming a contrasting image between the blood vessel through which the blood flows and the static environment that makes up the surrounding tissue (Figure 1).

To obtain such data, several algorithms have been developed by different manufacturers, making the images different in interpretation in order to distinguish them. Each OCT-A apparatus has its own algorithm, which is based on special experiential protocols, which are made to offer optimal information for appropriate clinical indications. Such variations in the interpretation of different devices can lead to different interpretations of clinical diagnoses [2, 3].

Monitoring changes in glaucoma today is easier and simpler than in the past. A number of new imaging techniques make this easier. OCT-A is just one such imaging technique. It is easy to monitor blood flow in the retina, while providing insight into the condition of ganglion cells that can be damaged by the disease itself. The popularity of using OCT-A to monitor changes in the optic nerve, and thus to monitor glaucoma changes, is growing. OCT-A is useful in assessing optic nerve perfusion in glaucoma eyes, since the density of peripapillary and macular blood vessels is impaired in patients with preperimetric glaucoma. This gives enthusiasm about the role of OCT-A in the detection of early changes in glaucoma. Quantitative data on retinal circulation have proven useful in analyzing the metabolic activity of the inner layers of the retina, and thus monitoring the disease itself. OCT-A also serves as a useful machine in monitoring blood flow (retrobulbar circulation) and detecting disease mechanisms in

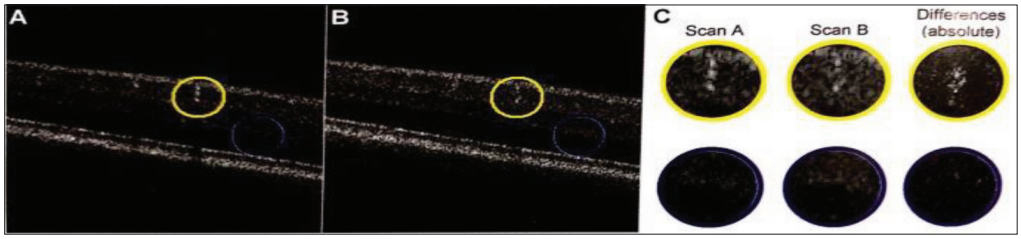

Figure 1. Display of the change in the intensity of the standard optical coherence tomography (OCT) signal and the optical coherence tomography with angiography (OCT-A) signal, as a function of the flow time (private collection); example of how the OCT signal intensity changes over time, after bulk motion correction; **A, B:** structural OCT images were acquired with a time difference of 8 ms; the location of a larger blood vessel (yellow circle) and of static tissue (blue circle) is indicated in both images; **C:** upon magnification of these areas and calculation of the differences, larger OCT signal changes can be seen within the blood vessel when compared with the static tissue; note that this figure is not showing the SPECTRALIS OCTA algorithm results, but just the absolute differences between two single OCT scans (A and B) for illustration

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Correspondence to:
Marija BOŽIĆ
Pasterova 2
11000 Belgrade
Serbia
ammilovic@gmail.com

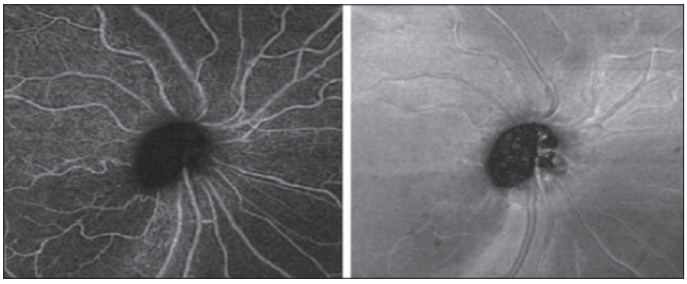

Figure 2. Optical coherence tomography with angiography (OCT-A) (left) and *en face* image (right) of a patient with glaucoma, in whom there is a lower temporal loss of the nerve fiber layer, with an anatomical correlation with a decrease in vascular density on OCT-A (private collection)

glaucoma without elevated intraocular pressure (normal-tension glaucoma) (Figure 2).

BLOOD FLOW AND GLAUCOMA

The very idea of detecting and monitoring glaucoma changes by measuring blood flow is not new. For decades, measuring retrobulbar circulation with various methods has been challenging in diagnosing and monitoring glaucoma. Fluorescein angiography, in glaucoma eyes, showed decreased fluorescence and delayed filling of blood vessels. Because it is invasive and only partially quantitative, fluorescein angiography is not practical for routine glaucoma monitoring. Other techniques, such as ultrasound Doppler, Doppler OCT, laser Doppler flowmetry, and laser speckle flowgraphy, have also shown the reduction in retinal and optic nerve flow in glaucoma. However, these techniques have high variability of measurement and are more suitable for detecting changes/differences between larger groups of patients than individually. OCT-A differs from the aforementioned techniques in two important aspects. Firstly, OCT-A is a non-invasive imaging technique. Secondly, OCT-A can measure the density of blood vessels with a high possibility of recurrence and renewal, i.e. reproductions of the measurement itself. It lasts only a few seconds and can be repeated at each patient checkup. Measurements are accurate and can be used to diagnose and monitor the disease.

HOW DOES OCT WORK?

OCT-A simply detects blood vessels to the level of capillaries. OCT-A software compares sequential frames of OCT images in cross section at the same position, looking for signal fluctuations that indicate blood flow. We have developed an algorithm called split-spectrum amplitude-decorrelation angiography – SSADA for short – which is so efficient that only two frames are needed to accurately identify capillaries [4]. This efficient algorithm, along with improvements in the Fourier velocity of the OCT domain, made OCT-A clinically feasible. As OCT-A senses movement, it is susceptible to artifacts created by eye movement, such as micro-saccades, eye pulsations, and eye twitches. Therefore, the production of high-quality OCT-A images requires special algorithms that monitor, process and reproduce this. These technical requirements have encouraged the rapid advancement of commercial OCT technology on the hardware and software fronts.

HOW TO DIAGNOSE OCT-A GLAUCOMA

In a 2014 OCT-A study measuring blood flow in glaucoma patients, Jia et al. found that glaucoma patients had reduced capillary density and blood flow index, at the level of disc superficial tissue and at the level of lamina cribrosa [5].

Elevated IOP may affect perfusion through the head of the optic nerve and deficiency of blood flow through the lamina cribrosa. The head of the optic nerve is not an ideal target for OCT-A imaging, given the shadow created by large blood vessels and the variability of the geometry of the rim of the disc and blood vessels. The focus of the imaging, for ideal imaging for the diagnosis of glaucoma, has been shifted to the peripapillary retina and macula [6, 7]. In both regions, the density of blood vessels showed precision and accuracy! In addition, the density of blood vessels showed a good correlation with the parameters of the visual field, even better than the correlation with the structural parameters of the OCT, such as the thickness of the layer of nerve fibers. It is important to note that a scan of sufficient size is necessary for the OCT-A image to have a useful diagnostic value. For the peripapillary region, for example, 4.5 × 4.5 mm scans have the best diagnostic usability. For the macular region, the most accurate is the 6 × 6 mm scan (Figure 3). There are studies that used smaller OCT-A scans, of 3 or 4 mm, but the accuracy was poorer, which is to be expected, since glaucoma first affects the peripheral parts of the macula.

BLOOD FLOW OR BLOOD VESSEL DENSITY?

In functional glaucoma tests, blood flow volume was first examined by Doppler OCT [8], and later by flow index by OCT-A [5]. The reproducibility of these measurements was poor. The reason may lie in the fact that the speed and volume of blood flow are affected by variations in the physiological state of the patient. These measurements have been shown to be influenced by the oxygen concentration in the inhaled gas mixture and visual stimulation. There are many other factors that affect blood flow. The reproducibility of measuring blood vessel density with OCT-A is excellent. Density is measured by direct (*en face*) reading of OCT-A images of appropriate anatomical structure. Maximum blood flow within the blood vessel itself converts a three-dimensional OCT-A finding into a two-dimensional direct (*en face*) angiogram. Blood vessel density is defined by the percentage occupied by vascular pixels (pixels with a higher flow signal relative to the non-vascular background) in the corresponding area in the *en face* angiogram. Large blood vessels can be ruled out if we want to measure the density of capillaries. For the diagnosis and monitoring of glaucoma, the density of blood vessels and capillaries is ideal.

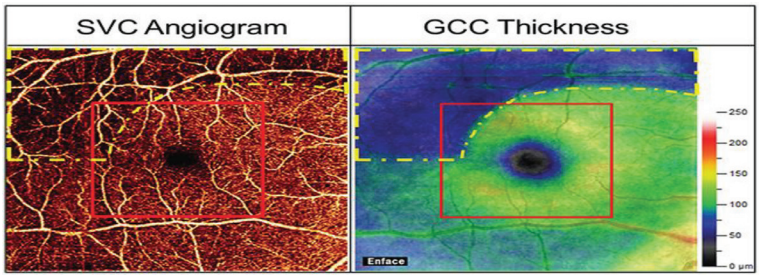

Figure 3. Superficial vascular complex (SVC) 6 × 6 Optical coherence tomography with angiography (left) and 6 × 6 mm layer of ganglion cell layer (GCL) thickness (right), in a typical glaucoma eye; glaucoma damage (yellow dashed line) is mostly outside the central 3 × 3 mm (marked with a red square); obviously, a larger area needs to be scanned for early detection of glaucoma damage (private collection)

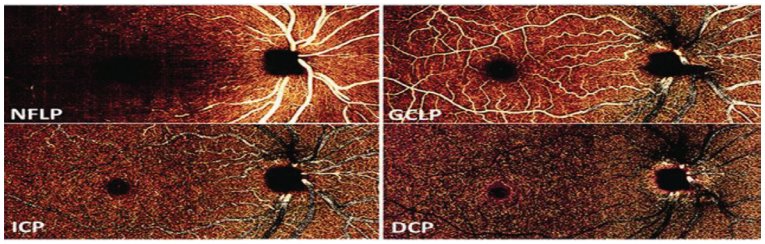

Figure 4. *En face* optical coherence tomography with angiography images of four retinal vascular plexuses, in the right eye, in a healthy person; nerve fiber layer plexus (NFLP) and ganglion cell layer plexus (GCLP) form the superficial vascular complex, which contains both large blood vessels and capillaries; intermediate capillary plexus (ICP) and deep capillary plexus (DCP) form a deep vascular complex, which is primarily formed by capillaries (private collection)

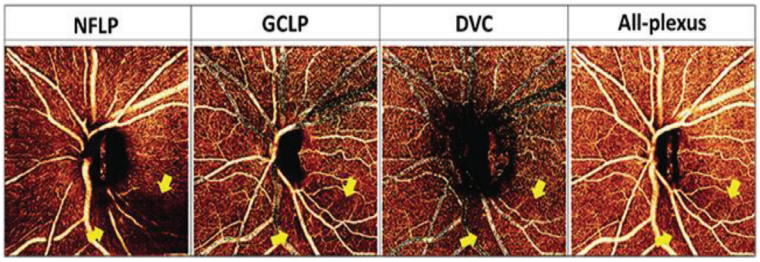

Figure 5. Image of a glaucoma patient, AngioVue optical coherence tomography with angiography apparatus (Optovue, USA), using a 4.5 × 4.5 mm AngioDisc scan; drop capillary loss, marked by arrows, can be more easily detected in the nerve fiber layer plexus (NFLP) than in the ganglion cell layer plexus (GCLP) and all plexuses (ALL-plexus), while the deep vascular complex is unaffected (private collection)

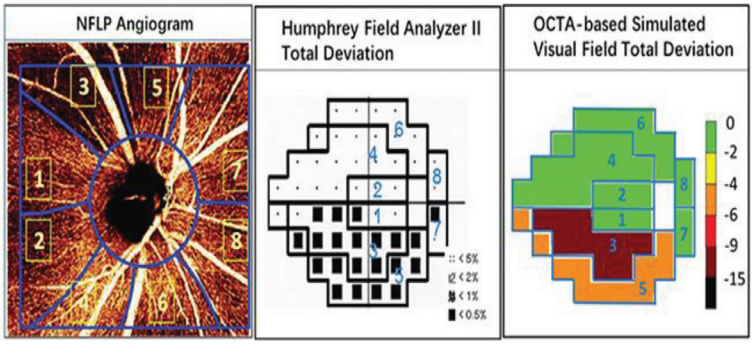

Figure 6. In eyes with moderately advanced glaucoma, *en face* optical coherence tomography with angiography (OCT-A) nerve fiber layer plexus (NFLP) and visual field map are divided into eight corresponding sectors, according to the Garway-Heat scheme; NFLP angiogram shows loss of capillaries (dropout) in the upper temporal part; on the OCT-A simulated field of view deviation map, sectors 3 and 5 show medium to advanced defects, which correlate with defects in the standard field of view (private collection)

WHICH VASCULAR PLEXUSES ARE AFFECTED

Earlier studies had limitations in the detection of blood vessels, since the shadow of the superficial ones blocked the deeper blood vessels. This has been overcome by developing an algorithm that distinguishes the *in situ* from the projected flow. OCT-A angiography, using this algorithm, can distinguish up to four vascular plexuses on the retina [9] (Figure 4). In the peripapillary region, glaucoma primarily affects the plexus of the retinal nerve fiber layer (NFLP), which feeds the retinal nerve fiber layer (NFL) (Figure 5). In the macula, glaucoma also affects the NFLP and the plexus of the ganglion cell layer (GCLP), which feeds the ganglion cell layer (GCL). Together, NFLP and GCLP form a superficial vascular complex. Focusing on peripapillary NFLP and macular superficial vascular complex (SVC) increases the visualization of glaucomatous lesions and improves diagnostic accuracy. It is shown that the density of macular SVC blood vessels has better diagnostic accuracy than the density of all plexuses in the retina [10].

WHY USE OCT-A?

According to the IRIS registry of the American Academy of Ophthalmology (AAO), OCT has already surpassed KVP in annual use for the diagnosis and monitoring of glaucoma. OCT monitoring of peripapillary NFL and macular ganglion cell complex are part of the standard examination in glaucoma. Measurement of OCT-A peripapillary NFLP vascular density is highly related to NFL thickness, and macular SVC density is highly related to GCC thickness. OCT plays a role in both early and late stages of glaucoma. Two clinical studies have shown that OCT-A can detect early preperimetric glaucoma better than structural OCT [11, 12]. Because OCT-A detects both dysfunctional (sick) and lost (dead) ganglion cells, while structural OCT detects only lost ganglion cells. In the early stages of glaucoma, diseased, dysfunctional ganglion cells have a reduced metabolism, which leads to a reduced density of capillaries. This reduced density is detected by OCTA. Prior to apoptosis, these ganglion cells lead to thinning of the NFL and GCC, which can be detected by structural OCT. By adding OCT-A to the diagnosis, we can detect glaucoma earlier and start treatment on time. Other studies have shown

that OCT-A parameters correlate better with visual field parameters compared to structural OCT parameters such as NFL thickness [6, 12]. This is partly due to the reduction of the "floor effect" by OCT-A measurements. The floor effect describes the fact that, although NFL thickness correlates with mean field deviation in early glaucoma, it shows low values in moderate glaucoma and then no longer decreases in advanced glaucoma. This limits the usefulness of NFL thickness for monitoring the progression of glaucoma in the moderate and advanced stages [13]. Although the density of blood vessels also eventually reaches the bottom, it appears to do so only in advanced glaucoma. OCT-A has the potential to improve glaucoma control in the moderate to advanced stages.

OCT FIELD OF VIEW SIMULATION

A method of sector simulation of the visual field using peripapillary NFLP OCT-A results has been developed (Figure 6). The results show a good correlation in the early and moderate stages of the disease. Simulated sectors of the field of view can also be summarized in the mean deviation parameter: NFLP_MD. NFLP_MD correlates with VF_MD, but has better diagnostic accuracy and reproducibility. Thus, a simulated field of view may be better than an actual field of view for monitoring glaucoma progression – in addition to being painless and more convenient.

Visual field testing is not only uncomfortable, but can result in unnecessary surgery or delayed treatment and vision loss. Statistical analyses showed that glaucomatous eye with MD deterioration at -1 dB / year needs testing every six months for four years to make the trend statistically significant (90% strength, $p < 0.05$), with a mean the deviation was already 4 dB worse [14, 15, 16]. This represents a 60% loss of retinal sensitivity, so the doctor has solid evidence and intensifies treatment. A more reproductive and objective method of monitoring the progression of glaucoma could detect significant progression earlier, thus enabling timely intervention to preserve vision.

AVAILABILITY OF OCT-A

AngioVue, which runs on the Avanti OCT system (Optovue, Inc, Fremont, CA, USA) has a good set of criteria for quantifying peripapillary NFLP and macular SVC [17]. On AngioVue peripapillary scans, NFLP is called radial peripapillary capillary (RPC). For the diagnosis and monitoring of glaucoma, high-resolution OCT-A scans of 4.5 × 4.5 mm for disc area and RPC density are recommended. Six-by-six-mm HD retinal surface density scanning should be used to scan AngioVue macula. The current AngioVue retinal surface plate corresponds to SVC. Zeiss's CIRRUS HD SEP platform has AngioPlek OCT-A software. AngioPlek 6-mm is used to scan the macula in the assessment of glaucoma. This system can analyze the surface density of the retina, which is called the perfusion density. This system does not currently have special software to analyze peripapillary NFLP around the disc. It is probably possible to export the images and process them for that purpose [18, 19]. Heidelberg has the OCT angiography module for Spectralis, which was introduced at the AAO meeting in 2018. Topcon has developed a fast-source SEP angiography software called OCTARA (under FDA approval) that runs on its Triton OCT platform.

CONCLUSION

The possibilities of OCT-A for glaucomatologists will expand in the near future. The possibility of detecting and monitoring glaucoma with this technology will also be expanded. All of these systems will undoubtedly offer software updates, making it easier for examiners to use the device itself, and thus monitor the disease. OCT-A represents the future in the diagnosis of retinal diseases and glaucoma.

Ethics: The manuscript has been written in accordance with the ethical standards of the respective institution and the journal.

Conflict of interest: None declared.

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Оптичка кохерентна томографија са ангиографијом код глаукома

Иван Марјановић[1,2], Весна Марић[2,1], Марија Божић[1,2]

[1]Универзитет у Београду, Медицински факултет, Београд, Србија;
[2]Универзитетски клинички центар Србије, Клиника за очне болести, Београд, Србија

САЖЕТАК

Оптичка кохерентна томографија са ангиографијом је неинвазивна имиџинг техника која омогућава тродимензионалну визуализацију перфузије кроз васкуларну мрежу ретине и хороидеје.

У блиској будућности ће се проширити могућности оптичке кохерентне томографије са ангиографијом за глаукоматологе. Проширеће се и могућност откривања и праћења глаукома овом технологијом. Сви ови системи ће несумњиво понудити ажурирања софтвера, олакшавајући испитивачима саму употребу апарата, а самим тим и праћење болести. Оптичка кохерентна томографија са ангиографијом представља будућност у дијагностици болести ретине и глаукома.

Кључне речи: глауком; оптичка кохерентна томографија; ангиографија

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CURRENT TOPIC / АКТУЕЛНА ТЕМА

Vaccination – a dilemma for a pediatric anesthesiologist: When is the right moment?

Vesna Stevanović[1,2], Gordana Kovačević[1,2], Marina Boboš[3], Predrag Stevanović[2,3]

[1]Dr Vukan Čupić Mother and Child Health Care Institute of Serbia, Belgrade, Serbia;
[2]University of Belgrade, Faculty of Medicine, Belgrade, Serbia;
[3]Dr Dragiša Mišović – Dedinje Clinical Hospital Center, Belgrade, Serbia

SUMMARY
Possible immune system interactions due to vaccination and drugs used in general anesthesia represent a dilemma for pediatric anesthesiologists in everyday practice. Immunosuppression caused by anesthesia and surgical trauma can affect the immunization process and cause-specific unwanted reactions. On the other hand, side effects due to vaccination can confuse clinicians in the immediate postoperative course. Both the nature of the vaccine and the type of surgery determines the delay period of elective surgical intervention. This current topic aims to present the scientific facts about the complex interactions between vaccination, immunization, general anesthesia, and surgical trauma and to provide recommendations for preoperative preparation.
Keywords: anesthesia; children; immunization; vaccines

INTRODUCTION

Vaccination causes the host's desired and controlled immune response to prevent the development of certain diseases and their complications. The schedule of mandatory vaccinations is most frequent in childhood [1]. At the same time, in children of all ages, it is sometimes necessary to perform a surgical intervention under anesthesia. Medicines and complex anesthesia processes also result in specific immunomodulatory functions, while surgery causes tissue trauma [2]. The vaccine is known to cause side effects similar to the stress response after surgery [3, 4]. On the other hand, scientists are trying to determine if the impact of the anesthetics changes the vaccine's effectiveness. That is why there are still preoperative dilemmas among pediatricians, anesthesiologists and surgeons: whether and when to vaccinate a child before surgical intervention, whether to postpone surgery because of vaccination, and when it is safe to continue with regular vaccinations after surgery.

The recommendations of the World Association of Pediatric Anesthesiologists differ regarding the delay of surgery when it comes to live attenuated vaccines [5]. The consensus has been made for inactivated vaccines. Interestingly, in recent years, the possibility of intraoperative administration of influenza and pneumococcal vaccines to specific pediatric patients during general anesthesia further complicates the abovementioned dilemmas [6].

FACTS ABOUT IMMUNIZATION

Immunization of the organism is carried out actively and passively. The active method involves using vaccines against a specific infectious disease, and the passive process involves using a defined immunoglobulin, antibody or serum. Currently, there are about 20 vaccines with which people can be immunized. The World Health Organization estimates this revolutionary medical discovery saves 3.5–5 million lives yearly [7]. It has been scientifically confirmed that individual variations in the strength of the immune response can have consequences on the protective efficiency and length of protection of the organism against a particular infectious disease. Factors affecting individual immunization variations are sex, genetics, comorbidities, and age-related factors [8, 9]. External factors include infections, antibiotics, probiotics, and previous vaccinations.

Habits such as smoking, alcohol use, stress, sleep quality, nutritional status, and exposure to toxins contribute to these complex processes. The most crucial factor in forming immunity is the vaccine itself (type of vaccine, dose, vaccination calendar, site of application, route of administration and co-administration of vaccines) [10]. Administration of drugs at the time of vaccination raises the question of vaccine effectiveness. These can be drugs the patient takes due to a chronic disease, but also analgesics and antipyretics that are recommended due to the side effects of vaccines [11]. In daily clinical work, pediatric anesthesiologists face preoperative dilemmas as to whether anesthesia drugs, the anesthesia process, and surgical

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Correspondence to:
Vesna STEVANOVIĆ
Dr Vukan Čupić Mother and Child Health Care Institute of Serbia
Radoja Dakića 6–8
11070 Belgrade, Serbia
vesna.stevanovic@med.bg.ac.rs

trauma affect the immunization process in the body in the case of vaccination and vice versa. It is estimated that a sixth of children operated on are at the age when, according to the national schedule, it is necessary to administer the vaccine [12]. The most significant number of postponements of elective surgeries occurs at the age of up to six months of life. The solution to this problem is better coordination and harmonization of positions on the issue of vaccinations between pediatricians, surgeons and anesthesiologists. An individual approach to the patient is recommended.

FACTS ABOUT ANESTHESIA AND IMMUNE RESPONSE

It has been scientifically proven that surgical intervention and general anesthesia cause a disturbance in the activity of the immune system. There are several explanations for this statement: the stress response with the release of catecholamines and cortisol suppresses macrophage function, phagocytic activity of leukocytes, phagocytic mobilization, complement fixation, lymphocyte transformation, T cell activity and antibody formation 4–12 days after surgery. It has been proven that anesthetic drugs are immunomodulators. Intraoperative events such as pain, hypoperfusion, hypothermia, hyperglycemia, transfusion or extracorporeal circulation also have an immunosuppressive effect. At the same time, there is an increased production of proinflammatory cytokines [13]. The degree of immunosuppression depends on the extent of the stress response, surgical trauma, perioperative pain therapy and infection. An immunocompetent child usually has no clinical manifestations of laboratory signs of transient immunosuppression due to anesthetic effects. Drugs for anesthesia have an immunomodulatory effect by changing the components of humoral and cellular immunity. Volatile anesthetics (halothane, sevoflurane, isoflurane, enflurane) inhibit the production of proinflammatory cytokines, the proliferation of lymphocytes, the activity of neutrophils, and natural killer (NK) cells, and the generation of free oxygen radicals while they do not affect the function of B lymphocytes. This condition can last for several days after exposure to volatile anesthetics. Nitrous oxide gas inhibits the activity of monocytes to the extent that it does not increase the risk of infection after surgery. A similar effect is shown by midazolam, ketamine, and fentanyl [14, 15, 16]. Thiopentone sodium reduces lymphocyte production and NK cell function. Propofol inhibits neutrophils, macrophages, and monocytes and increases infiltration of the operative wound by NK cells and helper T cells [17]. With its immunosuppressive effect, propofol does not affect the humoral immunity components. The benzodiazepines (lorazepam) show a more significant impact on the innate immune response. Studies have shown that dexmedetomidine and clonidine do not affect neutrophil function [18].

Opioids have a suppressive effect on the immune system, especially morphine and fentanyl, in contrast to alfentanil, remifentanil, and sufentanil. Unlike opioids, tramadol has a stimulating effect on NK cells [19]. Local anesthetics blocking sympathetic activity do not show an immunosuppressive effect [15, 17]. Transient neutropenia and lymphopenia are most often registered after surgical intervention and general anesthesia in children [20]. Clinical manifestations of the stress response after surgery are mild in the form of agitation and restlessness, elevated temperature, rash, pain, and rarely inflammation and sepsis. However, some works assume that the influence on the immune response due to anesthesia could increase the susceptibility to infection and even the spread of malignant tumors [21]. Cases of reactions after anesthesia in recently vaccinated children in the form of intussusception (after rotavirus vaccine), hypotonic crisis (after diphtheria, pertussis, and tetanus vaccine) and spread of infection in surgically immunocompromised patients originating from the live attenuated vaccine (oral polio vaccine, which is genetically unstable) have been described [22]. On the other hand, there is a justified fear that anesthetic drugs will change the immune effect of the vaccine if the anesthetics have already caused a certain degree of immunosuppression. Human studies have shown that the production of antibodies to anti-tetanus toxoids is lower if the child has been exposed to general anesthesia and surgery [23].

Also, fever, soreness at the application site, coryza, tearfulness, vomiting and malaise can be attributed to vaccination and surgical stress, which can confuse the clinician. Usually, these symptoms appear in the first 24–48 hours after vaccination, with the inactive form in 20% of vaccinated children and up to three weeks after the live attenuated vaccine (in 6% of cases and after the first dose of the vaccine). There are rare forms of severe post-vaccination complications, such as mumps meningitis (incidence 1:300,000 in vaccinated children) [24].

RECOMMENDATIONS OF THE WORLD ASSOCIATIONS OF PEDIATRIC ANESTHESIOLOGISTS

World Associations of Pediatric Anesthesiologists recommend delaying elective surgery one week after the inactivated vaccine and three weeks after the live attenuated vaccine (measles, mumps, and rubella vaccine – MMR, oral polio, tuberculosis vaccine) [23]. The Association of Pediatric Anesthesiologists and Intensivists of Serbia has the same stance [1]. A recent research has influenced the Association of Pediatric Anesthesiologists of Great Britain and Ireland, which, since 2021, advises postponing elective surgery 48 hours after administration of an inactivated vaccine (DTP, *Haemophilus influenzae* type B vaccine – Hib, meningitis type C, poliovirus). At the same time, they do not recommend postponing live attenuated vaccines surgical interventions (provided that the child is in good health) [25]. After oral vaccination with the live attenuated poliovirus, elective surgery should be postponed for 30 days [26]. The planned vaccination should be delayed for at least seven days if the patient had general anesthesia and surgery. However, if the child received blood or blood

derivatives during surgery and perioperative treatment, there are special warnings when planning to continue vaccination for MMR and varicella. If the patient received erythrocytes, the interval for postponing vaccination is up to five months; whole blood – six months; immunoglobulins for the treatment of idiopathic thrombocytopenic purpura – 8–11 months; plasma or platelets – seven months; tetanus immunoglobulin – 3–5 months; varicella immunoglobulin – five months; cytomegalovirus immunoglobulin – six months; hepatitis B immunoglobulin – three months; normal human immunoglobulin as hepatitis A prophylaxis – three months and normal human immunoglobulin as measles prophylaxis – 5–6 months [27]. Particular caution is required in children with congenital heart defects after surgical correction and extracorporeal circulation [28]. In these circumstances, the vaccines against measles, mumps, and rubella (the MMR vaccine) and the varicella vaccine are not recommended for the next seven months. If there is a need for low-dose aspirin after cardiac surgery, the aspirin should be continued, and the child should be vaccinated against varicella without delay. If cardio surgery is planned, the child can be vaccinated seven days before the intervention or 4–6 weeks after the surgery. Children after Norwood's operation, especially those born prematurely, are at high risk of vaccination because they may have sudden systemic and peripheral vascular resistance changes as an adverse event. Therefore, these children should be hospitalized after vaccination and observed for 72 hours [29]. If a splenectomy is planned, it is necessary to vaccinate the child at least two weeks before the operation with the meningococcal, pneumococcal, and Hib vaccines. Studies in adult patients have shown an enviable growth of antibodies to the mentioned vaccines in the case of urgent splenectomy if the vaccines are given immediately after the operation [30]. Vaccination against influenza and pneumococcus is increasingly encouraged after minor surgical interventions and diagnostic procedures under analgosedation during hospitalization or hospital discharge, especially in uncooperative or developmentally disabled children [12]. If the child is at an age when immunization is frequent (up to six months), it is better to postpone elective surgery at that time for older age. There is evidence that any delay in vaccination is harmful because an unvaccinated child is exposed to a high risk of developing an infectious disease, and the collective is at risk of an epidemic. The vaccination dilemma does not exist when it comes to emergency surgical interventions. In the case of co-administration of the vaccine and anesthesia drugs in those circumstances, careful patient monitoring is required for unexpected symptoms, especially after complicated and significant operations and long postoperative recovery. A special request is a vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease-2019 (COVID-19), currently not recommended for children aged under six months [31]. For now, this vaccine is not given in co-administration with other vaccines. A one-week gap is required between vaccines of different types. Also, a seven-day break is recommended after surgery until the vaccine is administered [25].

CONCLUSION

Vaccination is a revolutionary discovery in medicine that saves millions of lives every year. The reasons for postponing the planned vaccination should be precisely defined and reduced to a minimum. Postponement of vaccination or elective surgery is necessary if they overlap in time because both medical interventions have an immunosuppressive effect. The appearance of side effects in both cases can confuse diagnostic and therapeutic decisions.

Ethics: The manuscript has been written in accordance with the ethical standards of the respective institution and the journal.

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Вакцинација – дилема за педијатријског анестезиолога: када је прави тренутак?

Весна Стевановић[1,2], Гордана Ковачевић[1,2], Марина Бобош[3], Предраг Стевановић[2,3]

[1]Институт за здравствену заштиту мајке и детета Србије „Др Вукан Чупић", Београд, Србија;
[2]Универзитет у Београду, Медицински факултет, Београд, Србија;
[3]Клиничко-болнички центар „Др Драгиша Мишовић – Дедиње", Београд, Србија

САЖЕТАК

Могуће интеракције имуног система због вакцинације и лекова који се користе у општој анестезији представљају дилему за педијатријског анестезиолога у свакодневном раду. Имуносупресија изазвана процесом анестезије и хируршком траумом може утицати на вакцинални одговор организма и изазвати одређене нежељене реакције. С друге стране, нуспојаве због вакцинације могу збунити клиничаре у непосредном постоперативном току. Природа вакцине и врста оперативног захвата одређују период одлагања елективне хируршке интервенције. Циљ ове актуелне теме је да предочи научне чињенице о комплексним интеракцијама између вакцинације, имунизације, опште анестезије и хируршке трауме и да пружи важеће смернице за преоперативну припрему.

Кључне речи: анестезија; деца; имунизација; вакцине

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HISTORY OF MEDICINE / ИСТОРИЈА МЕДИЦИНЕ

Collaboration of Primarius Dr. Svetozar Živojnović and academician architect Nikola Dobrović in the development of medical infrastructure in Igalo

Andrea Raičević[1], Vladimir Stevanović[2], Marta Vukotić-Lazar[3], Dejan Tubić[3]

[1]University of Belgrade, Faculty of Philosophy, Belgrade, Serbia;
[2]Union – Nikola Tesla University, Faculty of Civil Engineering, Belgrade, Serbia;
[3]University of Priština – Kosovska Mitrovica, Faculty of Philosophy, Kosovska Mitrovica, Serbia

SUMMARY
Urban positioning, architectural conceptualization, and construction of health care facilities are parts of a complex process with a number of participants from fields of engineering, but also political and management structures on whose decisions the success of project implementation depends. In general, throughout history, the involvement of representatives of the medical profession in such endeavors has proven to be fruitful because in such a way they directly participate in the functional and formal design of their own work environment. In that sense, it can be stated that the collaboration of engineers, specifically architects, with medical doctors is all the more valuable for scientific research in the field of both the history of medicine and the history of architecture. This text deals with the implications of the collaboration between Primarius Dr. Svetozar Živojnović (1899–1981) and academician architect Nikola Dobrović (1897–1967) on the design and construction of several health care facilities, as well as the planning of urban zones in Igalo during the sixth and seventh decade of the 20th century. The paper emphasizes their respective contributions, which are considered crucial for the socio-economic development that has turned Igalo into a modern international health center, and one of the main centers of health tourism in the Mediterranean region.
Keywords: history of medicine; Svetozar Živojnović; Dr Simo Milošević – Igalo Institute; Nikola Dobrović; medical infrastructure

INTRODUCTION

This paper was written in the context of commemorating the 125th anniversary of the birth of academician Nikola Dobrović at the initiative of the Serbian Academy of Sciences and Arts (SASA), which declared the entire year of 2022 as the Year of Dobrović. The other dedication of the paper refers to the legacy of Dr. Svetozar Živojnović, which his son, Dr. Relja Živojnović (1931), a world-renowned ophthalmologist who spent his career in the Netherlands and Belgium, donated to the SASA Archives (2017), and which has been added to Dobrović's legacy in that institution [1].

The name of Primarius Dr. Živojnović is most often associated with the founding of the Adriatic coastal resort in Igalo, where he served for 13 years as the director (1949–1962). Dr. Živojnović was the first head of this institution, known for the use of medicinal mud and water called "Igalka" for therapeutic purposes. The institution was renamed in 1959 to Dr. Simo Milošević Institute for Physical Medicine and Rehabilitation and today it bears the name Dr. Simo Milošević Institute for Physical Medicine, Rehabilitation and Rheumatology. However, Živojnović's strenuous efforts to transform Igalo, a small coastal settlement in the westernmost part of the Bay of Kotor, where at the beginning of the 20th century there were "only 45 houses and 215 souls" [2], into one of the largest Adriatic centers for multidisciplinary rehabilitation, physical and preventive medicine, thalassotherapy, and spa-climatic treatment, have not been sufficiently explored. Therefore, Živojnović's role and significance in the history of medicine in these regions remain unclear.

Being active in the plans for the construction of Igalo, not only from a medical but also from an architectural-urbanistic aspect, Živojnović anticipated the development of this place into an international health center. Positioning the Institute as the nucleus of social and economic urbanization has created the need for planning the wider area of Igalo with an emphasis on the potentials of health tourism. Thanks to the mediation of Živojnović, the engagement in the preparation of this plan, as well as a series of individual health facilities, fell to his friend and relative with whom he spent his Prague student days, the renowned architect Dobrović.

As testimony to their friendship from that period, there remains Živojnović's portrait, the work of Nikola's brother, painter Petar Dobrović (1927), which is kept in the Živojnović family (Figure 1). As early as 1936, Dobrović and Živojnović jointly stayed in Herceg Novi for the purpose of designing "a hospital in the Bay of Kotor" as stated in the letter that Nikola sent to his brother Petar, and it is assumed that the text of the letter is referring specifically to the Igalo Sanatorium [1, 3].

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Correspondence to:
Vladimir STEVANOVIĆ
Savska 13A
11000 Beograd, Serbia
wladimirstevanovic@gmail.com

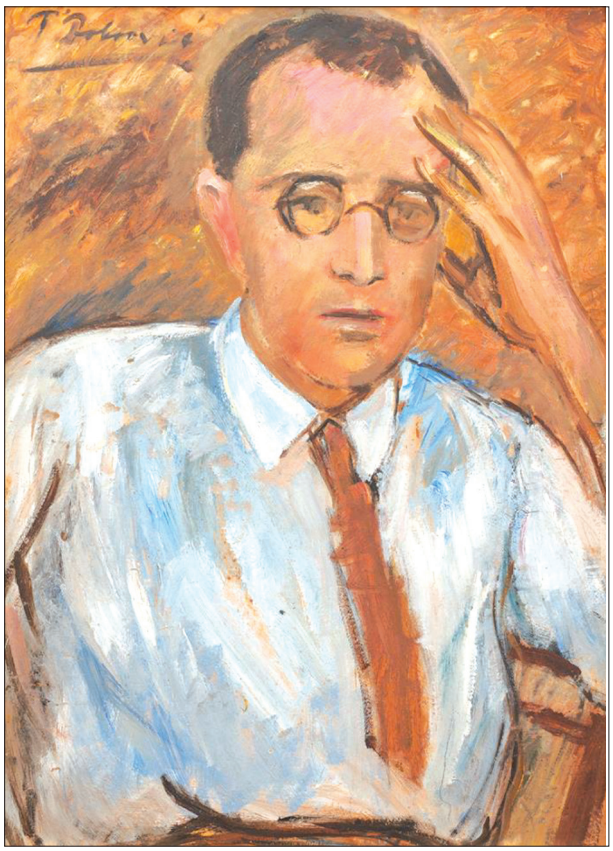

Figure 1. Petar Dobrović, Portrait of Dr. Svetozar Živojnović, oil on canvas (property of Dr. Relja Živojnović) (photography: Miloš Samardžić, 2022)

The intention of the authors of this paper is to present, by showcasing the aforementioned legacy, that it was precisely Dr. Živojnović who proposed to the Committee of Medical Experts – which was formed by the Secretariat for Public Health of the Federal Executive Council in Belgrade and made the decision to create the "Program for the development of a conceptual plan for establishing the Institute for Physiotherapy and Medical Rehabilitation in Igalo" – that the design be entrusted to Dobrović [4]. The paper will point to the importance of cooperation between architectural and medical professions, which has already been recognized in the academic community and represented in scientific research in the field of history of medicine [5]. It will also point to the fact that architect Dobrović, from the period of his student days in Prague, became a follower of a new spirit in health policy, which in his opus is reflected in the field of improving the building fund for the needs of social and medical institutions, as part of public urban equipment and policy [3].

THERAPEUTIC FACTORS AND APPLICATION OF IGALO MUD AND MINERAL WATER

The medicinal effects of Igalo mud and "Igalka" mineral water have been known for centuries through folk experience as well as numerous clinical studies, which have confirmed their benefits for a large number of patients treated at the Institute. The natural bounty of the Bay of Kotor, in the area between Herceg Novi and Igalo, geological-biological features, and climatic and physico-chemical factors, have been the source of the healing properties of sea mud and mineral springs. Igalo mud is marine silt originating in the coastal area of Topla Bay, whose healing properties are influenced by mild radioactivity, mixing and sedimentation of mineral deposits from the Sutorina River, sea water, and the involvement of specific marine flora and fauna, shallow water, solar radiation, soft soil, geohydrological and climatic factors. Indications for use include the treatment of rheumatic diseases – rheumatoid and psoriatic arthritis, ankylosing spondylitis, degenerative spinal and extremity joint conditions, extra-articular conditions, post-traumatic conditions, diseases and injuries of peripheral nerves, and chronic gynecological conditions such as adnexitis, parametritis, and secondary sterility. Therapeutic effects in clinical practice are prominently hyperemizing, resorptive, and anti-inflammatory, as well as spasmolytic and analgesic effects, and also have a positive impact on osteogenesis in bone fractures and on the regeneration of peripheral nerves after injury [6, 7].

As for the composition of the "Igalka" mineral water, it is primarily made up of sodium chloride (NaCl), hence it is referred to as sodium-chloride, saline, or muriatic water. Igalka is clear in appearance, colorless and odorless, salty in taste, and slightly alkaline, with negligible radioactivity. The water, with a source temperature of 14.8°C, has dilatory, spasmolytic, analgesic, and relaxant effects. It is used for mineral baths or combined with kinesitherapy in a pool or a butterfly-shaped tub (hydrogymnastics), with galvanic current (hydrogalvanotherapy), under air pressure, with mud (mud baths), then under increased pressure (Scottish showers), for underwater massage, with a gradual increase in water temperature, etc. The indicated scope for application includes chronic rheumatic diseases of joints, muscles, and connective tissue, peripheral nervous system diseases, conditions following trauma and surgeries on the locomotor apparatus, chronic gynecological and skin conditions (psoriasis). Internal use is done by drinking, which increases gastric juice secretion and motility of the digestive tract, and by inhalation, which acts secretolytically and is used for diseases of the digestive and respiratory systems. For chronic diseases of female reproductive organs, it is used in the form of vaginal sprays [8].

Mineral springs in Igalo were first mentioned in Austro-Hungarian military documents (1875). The first written document about Igalo mud was created by Dr. Rudolf Levi (1910), the head of the rural hospital in Trebinje, while international confirmation of its healing properties was provided by the laboratory of the renowned French spa Vichy (1930) [9].

FIRST CONCEPTS OF A HEALTH CENTER IN IGALO AT THE INITIATIVE OF DR. ŽIVOJNOVIĆ

Dr. Svetozar Živojnović was born on October 20, 1899, in Sombor. After completing secondary school in Novi Sad (1918), he studied medicine in Zagreb (1918–1919),

Prague (1920), Vienna (1920–1923), and Graz (1923–1926), where he graduated and met his future wife Milena Bubalo, who later became an ophthalmology specialist [6]. In 1921, he fell ill with tuberculosis of the retina and was almost blind for nearly a year. His wife greatly helped him so that his disability would not impede his medical career. After an internship in Novi Sad, Živojnović worked as a general practitioner in Vojvodina villages. He was then appointed as the physician for the State Dispensary for Tuberculosis in Herceg Novi (1934), while his wife became an ophthalmologist at the Military Hospital in Meljine [2].

Upon arrival in Herceg Novi, the mud in Igalo became the focus of Živojnović's interests, where he and his wife participated in scientific analyses of its medicinal properties. This research initiative was started by the Consul of the Kingdom of Yugoslavia in Milan, Dr. Dušan Marinković, along with the owner of the Igalo Hotel, Miloš Janković [10], a relative of Živojnović's wife, investors (industrialist Vlado Savčić and banker Dragomir Leko), and professors from the University of Belgrade (Dr. Milan Luković, Dr. Aleksandar J. Ignjatovski, Dr. Aleksandar Ščebrakov, and Dr. Dragoljub K. Jovanović, a colleague of Marie Curie). Based on their final analysis in 1938, the Faculty of Medicine in Belgrade issued a certificate of the medicinal properties of Igalo mud [9]. In addition to participating in analyses crucial for forming the idea of an Igalo spa, Živojnović anticipated the realization of Igalo's potential in terms of health tourism. He became the organizational secretary of the 4th Yugoslav Congress against Tuberculosis (Herceg Novi, May 1938), which brought together doctors from all over the country, with a series of accompanying programs, excursions, and entertainment for participants who were accommodated in the city's hospitality facilities [2]. This event represented the beginning of both congress and general health tourism in Herceg Novi, and Živojnović emerged as a key figure in this respect. In summary, it can be said that Živojnović's activities were crucial for achieving two significant visions of Igalo: establishing a new health center and transforming the town into a hub for regional health tourism, which will be discussed further below.

After a lull in activities related to the organization of the spa due to World War II, in 1949, the Minister of Health of Montenegro, Mato Petrović, at the suggestion of Vladimir Marinović (an official from the Protocol of the Ministry of Foreign Affairs of the FPR Yugoslavia and the brother of the aforementioned Dr. Marinović), contacted Živojnović to re-engage in the planning of a health center in Igalo [9, 11]. By a decision of the Government of Montenegro (1949), a balneological and climatic spa was established in Igalo, which was named Adriatic Natural Spa by a decision of the National Assembly of Montenegro [2]. Initially, therapy was performed on the muddy beach near the mouth of the Sutorina River, at a location called Stara Banja (Old Spa) (Figures 2 and 3). There, a peloid facility was built for the manipulation and storage of mud.

In one of the studies, Dr. Živojnović described the circumstances of the spa's founding: "There was only a wonderful southern coastal climate, with lush subtropical vegetation and fragrant and crystal-clear air, blue and salty sea, with flat sandy shores, through whose transparent water one could see black sediment on its bottom, already known to the people as therapeutic sea mud. Above the mouth of the Sutorina River, in the dense undergrowth of prickly mastic trees, one could hear the deep gurgling of the cold springs of Sutorinska Slatina, whose waters were lost in the nearby riverbed, and the inhabitants of the surrounding villages knew that these waters were medicinal. At the moment of founding, the spa had a small building of weak material, totaling about 40 m^2, and one entirely dilapidated woodshed. The first outpatient clinic and the spa office were placed in the building, and in the three remaining rooms, improvised "male and female wards" and "rest areas" were set up, each with four beds, where procedures with therapeutic sea mud were performed. The boiler room of this healing center was represented by one large gasoline can, placed on an iron tripod, under the open sky, where the "black medicine," i.e., Igalo mud, was heated. The waiting room for patients was replaced by a nearby mulberry tree, under whose shade, instead of benches, bare planks were placed on piles of stones. Patients would patiently wait there for their turn to be treated, and in the meantime, those who were still physically able would use shovels to collect and extract the therapeutic material from the shallow coastal areas, which was necessary for their own treatment as well as for the treatment of other patients. In that area, at that time, there was only one shallow well, which would dry up during the hot months, so it was necessary to bring water from more distant areas to facilitate washing patients after the mud procedures" [12].

After a discouraging start, a crucial step in the rise of the Adriatic coastal resort was the allocation of the nationalized Igalo Hotel to the healing center, whose pre-war owner was the aforementioned Miloš Janković – one of the initiators of establishing a new health center in Igalo. The spatial capacities of the hotel allowed for an expansion of 50 beds in a newly arranged stationary facility built on the site of today's main building, erected in the first phase of the Institute's construction. Then, new premises were built based on mud therapy and mineral water, and in 1955, the existing stationary facility of the former Igalo Hotel was expanded, increasing the number of patient beds to 105 [11]. The water shortage was solved by tapping four "Igalo" springs and drinking water from the source in Mojdež, through which water with a volumetric flow rate of 20 L/s reaches the healing center [8]. With the construction of a transformer station, a meteorological station, and an administrative building, the communal infrastructure for the functioning of the healing center was created. This translation aims to capture the essence and details of the original text as closely as possible. The presented data show the thorny path of the Adriatic natural healing center, initially a conventional spa using locally available natural remedies for treatment, and later a respectable medical institution that puts therapeutic natural goods under medical and scientific control, and employs them according to defined indication areas and appropriate physiotherapeutic methodology.

Figure 2. Igalo, Old Spa (source: SASA Archives, Historical Collection 14878/II, photo-documentation, Legacy of Dr. Svetozar Živojnović, a gift from his son Dr. Relja Živojnović to SASA Archives)

Figure 3. The first building of the Institute – Old Spa (source: SASA Archives, Historical Collection 14878/II, photo-documentation, Legacy of Dr. Svetozar Živojnović, a gift from his son Dr. Relja Živojnović to SASA Archives)

FOLLOWING DOBROVIĆ'S PROFESSIONAL INTEREST IN HEALTH FACILITIES

For Živojnović's vision, which involved developing Igalo into an international health center, an initial need was for a regulatory urban plan for both the immediate and wider area of the Health Center, to study the settlement's issues and its future purpose as a health center. The task of creating the plans fell to the architect Nikola Dobrović, whose biography we outline briefly due to space limitations [13], but we emphasize his rich experience in designing medical programs, which was one of the arguments for his engagement in Igalo.

Born in Pécs (1897), Dobrović began his architectural studies in Budapest (1915) and then, after an interruption due to World War I, continued at the Czech Technical University in Prague, where he graduated (1923). After his first job in Prague (1928–1929), he continued his career in the Mediterranean region on the island of Lopud, near Dubrovnik (1929–1941). After World War II, he became the Director of the Urban Institute of the People's Republic of Serbia (1945), Director of the Urban Institute of Belgrade (1946), a regular professor at the Faculty of Architecture in Belgrade (1947), and a regular member of the Serbian Academy of Sciences and Arts (1965).

From his student days in Prague (1919–1923), Dobrović turned to the topic of medical facilities, among which the most prominent are the student work Museum of Medicine (1920) and the graduation work Home for the Care of the Blind (1923). Also, Dobrović's first realized objects in the Prague period were the result of cooperation with healthcare workers (Villa Dr. Bulirzha and the building with the pharmacy of Mr. Jindrak). His most significant Prague endeavor was participation in creating the social institutions complex "Masaryk's Homes," which included nursing homes, shelters for children and adults suffering from incurable diseases, recuperation facilities for "weak" children, and recreation centers (1926–1928). In this work, Dobrović designed children's departments and the park environment of the complex [3]. The tradition of cooperation with doctors, Dobrović continued in Dubrovnik, designing the summer residences of Dr. Ivan Račić (1937), Dr. Marko Mladinov (1937), Dr. Vojislav Mitrović (1939), and Dr. Edgar Wolf Vuković (1939). It is assumed that Dr. Vuković and Dr. Račić, both otolaryngologists, contributed to the creation of Dobrović's project for the Ear, Nose and Throat Department of the new Banovina Hospital in Dubrovnik (1939). In this context, it is worth mentioning the project for Dubrovnik's Kursalon (1930), as well as participation in competitions for health facilities such as the Regional Hospital (Sarajevo, 1928), Banovina Hospital (Split, 1930), Zakladna, and Jewish Hospital (Zagreb, 1931) [3].

Dobrović relies on the climatic potential of locations in his design work, emphasizing the inseparable connection between the Mediterranean regions and the search for health. He addresses the movement of people from large cities seeking rejuvenation through the southern sun, clean sea air, and iodine-, chlorine-, and bromine-rich seawater, all of which contribute to their health and energy for further work and life struggle [14]. Dobrović was perhaps directed towards the typology of healthcare facilities not so much by the stylistic architectural current of modernism as by the social context of post-war modernist-progressive affirmation of healthcare architecture and the democratization of the right to healthcare.

PROJECT ACTIVITY AND CONSULTANT ROLE OF DOBROVIĆ IN RESOLVING ALL IMPORTANT URBAN DEVELOPMENT ISSUES OF HERCEG NOVI

Upon arriving in Herceg Novi at the initiative of Dr. Živojnović, Dobrović studied the archival material, technical and photo documentation, and collected topographical-geographical historical maps of the city and its surroundings, on the basis of which he published the book titled "Urbanism Through the Ages I – Yugoslavia" (1950) [15]. In this book, Dobrović highlighted the connections between natural and built dominants and established transit routes, facilitating an understanding of regional urban genesis. Preliminary research led to conclusions about the terrain properties of this part of the Bay of Kotor, which are summarized by factors of proximity (to the water surfaces of Toplanski Bay and Kumbor Canal; elongated stretches of coastal lines;

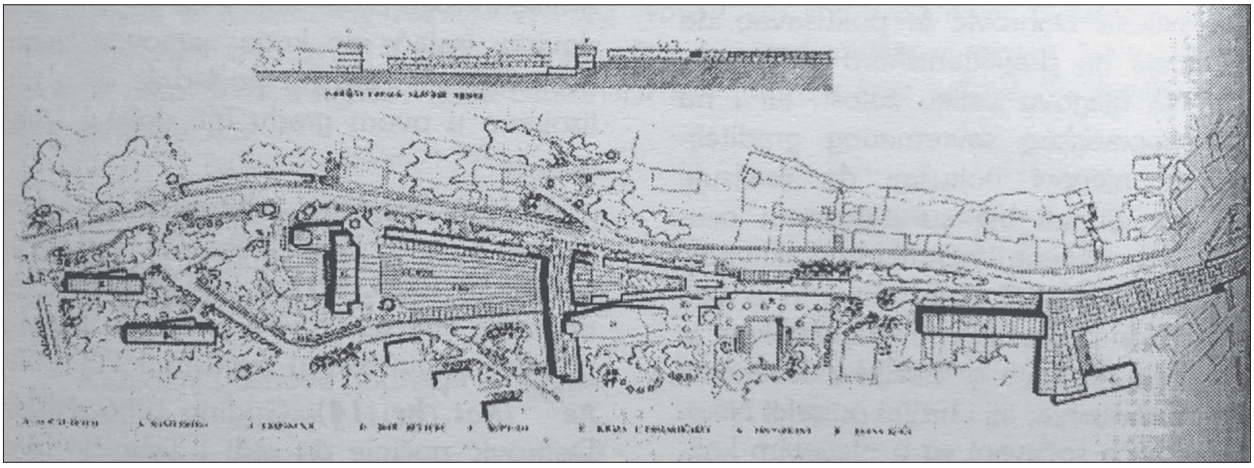

Figure 4. Plan of Herceg Novi center (source: SASA Archives, Historical Collection 14878/II)

flat terrains of the inter-hill regions of Igalo, Meljine, and Zelenika; beaches of Igalo and the terrain of Savina, Meljine, and Zelenika) and factors of distance (surrounding peaks and mountain ranges of distant areas). Favorable views and the inclination of the slopes of Savina justified the placement of hotel resorts, sanatoriums, and housing, while favorable vistas, clean air, and equidistance from all parts of the settlement affirmed the development of educational functions.

Dobrović's concrete work began with the study titled "Basics of the Urban Plan of Igalo" (1951), based on which the Directive Urban Plan for the Arrangement and Construction of Igalo as a Health Center and Tourist Place (1953) was completed [16]. The Directive Plan envisaged a rational zoning of Igalo's territory and a framework for the realization of a new healthcare center. This was an initial planning document with set guidelines for future elaboration. Dobrović then supplemented it with an Elaborate Preliminary Design (1956), which detailed three regions of Herceg Novi (areas of Sutorina and Meljine, Zelenika, and the center of Herceg Novi). The interconnectedness of these entities was achieved by relying on natural factors, bodies of water, and coastal lines, as well as on the structure of roads and the course of the Adriatic tourist road (today's Adriatic Highway), and the direction of the runway which defined the area between the Tivat and Grbalj fields. The conceptual elaboration was based on previously accepted Directive Plans of the mentioned areas. However, the Directorate for Roads of Montenegro subsequently adopted the project of the Adriatic Tourist Road, which conflicted with Dobrović's initial plans, affecting the documentation of the Preliminary Design to be made in two parts.

After 10 years of development and adjustment, the plan took its final form as a section of the Main Project for Urban Planning of the Municipality of Herceg Novi (1961) [17]. This Dobrović plan was the first systematic urban planning document in the history of Herceg Novi. It included project solutions for Igalo and the city center within the scope of 10 kilometers of the coast (Figure 4). The significance of the scope Dobrović defined by structuring its unique image as part of a larger system, in the regional framework of the Bay of Kotor and the Dubrovnik Archipelago. The scope of the plan was larger than that of the Directive Plan, and at a level of detail corresponding to the scale of 1:500, the plan provides for the character and manner of using the basic zones of the Herceg Novi part of the bay, directing the Adriatic Magistrala road, organization and arrangement of the city center towards land and sea, and the zoning arrangement of urban functions, particularly tourism and therapeutic services in Igalo [16]. Dobrović elaborated in detail the arrangement of the central city zone, which he conceived as a linear series of spatial entities and central content, such as local government buildings, the main pedestrian square, the Post Office building (1962, built according to the Dobrović's project), a cultural center, an extension of the building of the NO Municipality of Herceg Novi (1962, realized), public beaches, and the Boka Hotel with its accompanying park [18].

As early as 1959, Dobrović and Živojnović produced a photomontage of the projected facility of the Institute between Đurić's and Ćurić's hills, showing the commitment to a sloping building between two neighboring hills, in direct spatial and functional connection with the immediate natural context. As Dobrović himself pointed out, in this way the object synthesizes the advantages of block and pavilion systems of space organization (Figures 5, 6, and 7) [4, 18].

Urbanistic parameters have conditioned the determination of both the narrower and broader locations of the medical circle in Igalo, with designated positions for the Institute and the Children's Department [17]. Both locations were defined according to the proposal of Dr. Živojnović, based on the program for further development of the spa with 520 beds, which was drafted in 1959. Morphological characteristics of the terrain and climatic factors conditioned the scope of the intervention between the hills in the coastal area of Igalo. The spa's needs at the time were organized through zoning differentiation of purposes: accommodation and therapeutic units for patients, open terraces for physical exercise, gatehouses, boiler rooms, summer stages, tennis courts, basketball and gymnastics areas, swimming pools, sunbathing areas, commercial facilities, and traffic areas. The condition for the realization of this segment of the urban plan was the demolition of the old Institute building as an outdated and

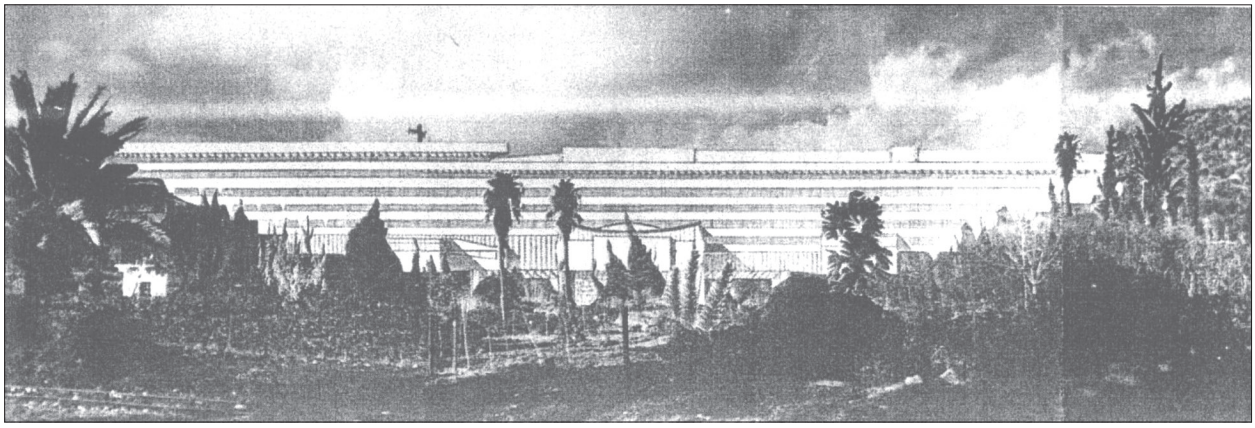

Figure 5. Nikola Dobrović, Location and photomontage of the facility of the Institute for Physiotherapy and Medical Rehabilitation in Igalo, black and white photography (source: Vukotić-Lazar M. Beogradski period arhitekte Nikole Dobrovića. Beograd: Plato; 2002. p. 95)

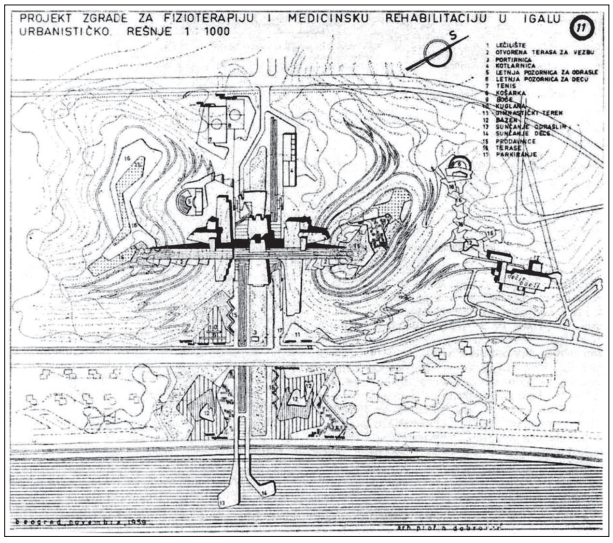

Figure 6. Urbanistic plan of non-built Institute (source: Dobrović, N. Zavod za fizioterapiju i medicinsku rehabilitaciju u Igalu. Zbornik radova Instituta za arhitekturu i urbanizam, 1961; I)

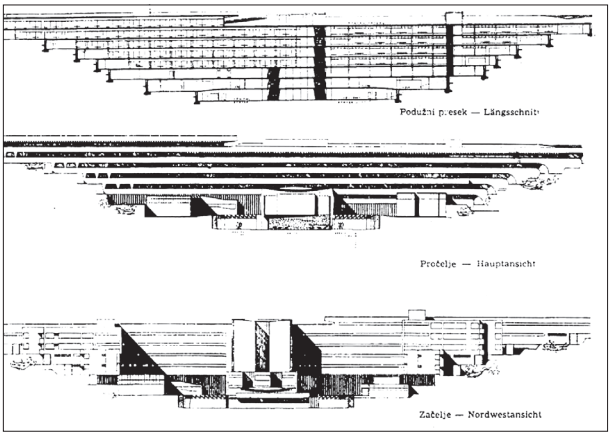

Figure 7. The facades of the non-built Institute (source: Dobrović, N. Zavod za fizioterapiju i medicinsku rehabilitaciju u Igalu. Zbornik radova Instituta za arhitekturu i urbanizam, 1961; I)

depreciated object (Old Spa). The urban concept of the Institute's prospective construction was considered by the expert commission of the Secretariat for Public Health of the Federal Executive Council and adopted in Belgrade (September 1959). The program concept of the new Institute building was presented to the Institute's management for expertise (December 1959); however, during the realization of the Program and the project of the Institute's Phase II (1982), this proposal was ignored and the Institute building was built on the basis of a different program task [16]. Despite dedication and detailed elaboration, Dobrović's visionary plan did not come to fruition. Namely, the projected scale of investment exceeded the financing possibilities. Not only was the plan not implemented, but it was also rejected as unrealistic. The conservative local community could not face modern urban standards, instead giving priority to economic potentials and problems. As Dobrović's sketches were displayed multiple times over an extended period in Herceg Novi, being the subject of public discussion, changed but never adopted, Dobrović reluctantly withdrew from collaboration with local services and abandoned the vision of Igalo's urbanization.

The failure and disrespect of the plan, on which he was a direct collaborator, motivated Živojnović to resign and take early retirement (1962), so that the Municipal Assembly of Herceg Novi (1966) proceeded with the preparation of a new and different urban plan covering the area from Igalo to Meljine [2]. Although he had ceased involvement in the development of Igalo after the collapse of Dobrović's plan, Živojnović tried again in 1967 to engage in these activities. In the year when a 'B' category annex with a capacity of 140 beds was built next to an existing hotel in Igalo (unrelated to Dobrović's plans), Živojnović published a documented program study titled "Physiatric Potentials of Igalo and Guidelines for Further Development and Construction of the Institute for Physical Medicine and Rehabilitation." In the study, he expressed disagreement with the construction of a new spa circle at the location of the object built in the first phase of the construction of the Institute, which still exists today [12]. The criticism referred to the new management of the Institute, which decided to build at the location in the center of Igalo, thus preventing the organic connectivity between the interior and exterior of the object. Close contact with the residential zone of Igalo led to the exposure of patients to the routines of everyday life of the surrounding population and modes of using spatial resources that are unrelated to the medical-balneological

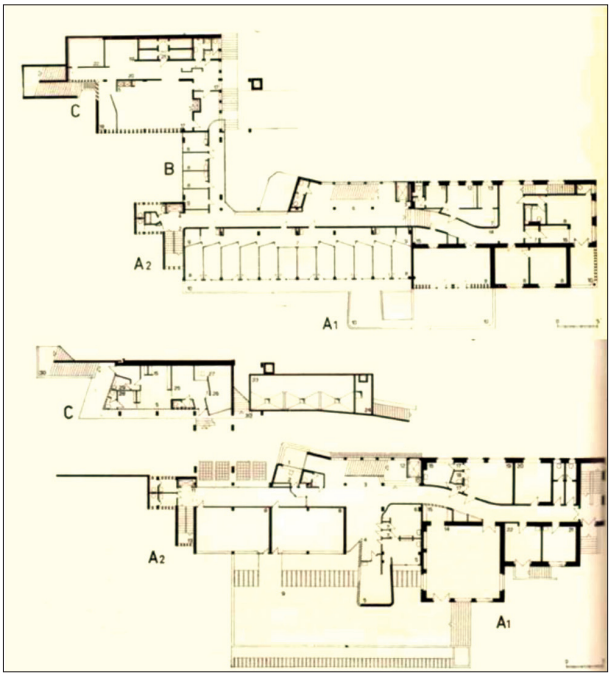

Figure 8. Ground floor and the first floor of the Children's department of the Institute (source: Babić Lj. Dečje odeljenje za fizikalnu terapiju u Igalu, Arhitektura urbanizam, 1967; 43)

center for rehabilitation. Population density and frequent use of space increased with the subsequent construction of a solitary settlement with a commercial zone, so the aforementioned shortcomings have not been eliminated even today.

BUILT INDIVIDUAL OBJECTS IN THE MEDICAL ZONE OF IGALO

Apart from the preparation of urban plans, most of which have remained only a testimony to the process of professional educated planning, Dobrović built several individual objects. In the early 1950s, he implemented the Entrance Gatehouse of the Institute, i.e., the "Gatehouse," which was demolished in the process of subsequent investment phases of construction (1972) [16].

The largest and most significant work of architect Nikola Dobrović in Herceg Novi is the Children's Department for Physical Therapy, created in the period 1959–1962 and located within the system of the then Institute in Igalo [19]. The building represents an extensive expansion and reconstruction of the former cable center. In line with the disposition of functions of the Institute's building that Dobrović planned, a series of design decisions concerning the Children's Department were implemented (Figures 8, 9, and 10).

The ground-level arrangement of the surrounding spaces included elements of the health complex into the natural environment. The position between two adjacent hills inevitably conditioned the spatial-functional connection of the building with the natural context. In this way, climatic, soil, and biotic factors, in agreement with social factors and the functional arrangement of the built environment,

Figure 9. Nikola Dobrović, Children's Department of Physical Therapy (photography: Andrea Raičević, 2022)

Figure 10. Nikola Dobrović, Children's Department of Physical Therapy (photography: Dragan Dragin, 2021)

contributed to the sustainability of the health prospects of the complex [16]. To emphasize the ground surfaces and their connection with the object, direct access to the ground level from all floors has been defined. According to the established model, individual buildings in the garden areas are framed by a network of pedestrian paths, open swimming pools, and rehabilitation devices, accessible from each floor of the building that provides functional connectivity and accessibility of the surrounding land. At the ground level, there are free zones for pedestrian passage with routes of continuous movement. The accommodation block, distributed over five floors, consists of patient rooms that are programmatically arranged. Contact with nature is ensured through balconies in all rooms, and physical activities (walks) for patients during bad weather are facilitated by long corridors. Corridor pathways continue onto pedestrian paths for greater comfort when transitioning from the indoor to the outdoor space. The roof plane is adapted for hydrotherapy treatment and activities under the open sky [18].

CONCLUSION

The paper discusses the respective contributions of Dr. Svetozar Živojnović and architect Nikola Dobrović to the development of Igalo's medical infrastructure and the

improvement of this urban area into a center for health tourism in the Adriatic region. Dobrović and Živojnović actively participated in the urban planning of the Herceg Novi municipality and foresaw the detailed development of a larger number of individual healthcare facilities.

Although guidelines for design, in the form of systematized needs for the city's development, were directed to Dobrović by various commission-regulatory bodies of the Public Committee of the Herceg Novi municipality, while professional communication was mediated by the City Council body, the significance of Živojnović's advisory role in these processes was crucial. Dobrović had a mutual relationship of respect and uncompromising trust with Dr. Živojnović. As an architect, he subordinated his personal interest to social responsibility, and in the desire to support Živojnović's efforts, delivered plans and projects at a minimal fee, sometimes even for free. During their 13 years of active cooperation, Živojnović and Dobrović detailed several projects, many of which remained at the idea level. However, the urban-architectural concepts of these projects were always grounded in progressive efforts for growth and development of this region, in health tourism, as well as in strong encouragement for overall societal progress and modernization.

Ethics: This article was written in accordance with the ethical standards of the institutions and the journal.

Conflict of interest: None declared.

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Сарадња примаријуса др Светозара Живојновића и академика и архитекте Николе Добровића у развоју медицинске инфраструктуре у Игалу

Андреа Раичевић[1], Владимир Стевановић[2], Марта Вукотић-Лазар[3], Дејан Тубић[3]

[1]Универзитет у Београду, Филозофски факултет, Београд, Србија;
[2]Универзитет „Унион – Никола Тесла", Факултет за градитељски менаџмент, Београд, Србија;
[3]Универзитет у Приштини – Косовска Митровица, Филозофски факултет, Косовска Митровица, Србија

САЖЕТАК

Урбанистичко позиционирање, архитектонска концепција и изградња здравствених објеката припадају сложеном процесу који укључује низ актера из инжењерске струке, али и политичко-управљачких структура од чијих одлука зависи успешност реализације. Генерално посматрајући кроз историју, инволвирање и представника медицинске струке у овакве подухвате показало се плодоносним јер они тиме директно утичу на функционално и обликовно осмишљавање сопственог радног окружења. У том смислу, може се констатовати да су сарадње инжењера, конкретно архитеката, са лекарима утолико вреднije за научна истраживања у области како историје медицине, тако и историје архитектуре. Овај рад се бави импликацијама сарадње примаријуса др Светозара Живојновића (1899–1981) и академика и архитекте Николе Добровића (1897–1967) на изградњи више здравствених објеката и планирању урбанистичких целина у Игалу током шесте и седме деценије XX века. Истичу се њихови респективни доприноси, који се сматрају кључним за друштвено-економски развој који је Игало претворио у модеран здравствени центар међународног карактера и један од главних центара здравственог туризма у медитеранској регији.

Кључне речи: историја медицине; Светозар Живојновић; Завод „Др Симо Милошевић – Игало"; Никола Добровић; медицинска инфраструктура

IN MEMORIAM

Сто година од смрти професора Војислава Ј. Субботића, родоначелника хирургије у Србији и првог редовног професора хирургије на Медицинском факултету у Београду (1859–1923)

Радоје Чоловић

Српска академија наука и уметности, Београд, Србија

САЖЕТАК

Ових дана (4. 12. 1923. по јулијанском, 17. 12. 2023. по грегоријанском календару) навршава се сто година од смрти родоначелника хирургије у Србији, једног од оснивача, и уз Милана Јовановића Батута првог (редовног) професора Медицинског факултета у Београду, првог професора хирургије, првог продекана и по редоследу другог декана, првог хирурга декана, оснивача и шефа Катедре хирургије, оснивача и првог управника X хируршке клинике, вишегодишњег одличног председника Српског лекарског друштва, организатора Првог састанка српских хирурга (1907) и Првог југословенског састанка за оперативну медицину (1911), најсвестранијег и најпризнатијег српског хирурга, чија су пионирска достигнућа у васкуларној хирургији деценијама остала непревазиђена. Познати амерички хирург Н. М. Рич и сар. су 1983. године у чланку „The Matas/Soubotitch connection", објављеном у познатом часопису *Surgery* написали следеће о његовим достигнућима: „Иронично је да је прошло скоро 40 година пре него што су слични успеси постигнути током другог дела Корејског сукоба (1952–1953)".

Кључне речи: Војислав Ј. Субботић; родоначелник свих грана хирургије; Србија

Породица Војислава Ј. Субботића

Војислав Субботић је рођен у Новом Саду у патриотској српској грађанској породици, од мајке Савке Полит (1834–1918), (сестре Михаила Полит-Десанчића) из грчке (цинцарске?) породице која се доселила У Нови Сад из Цариграда. Савка се школовала у Новом Саду и од 1848. када је породична кућа била порушена па до обнове куће школовала се у католичком интернату у Бечу. За Војислава Ј. Субботића удала се 1851. године. Савка Субботић је била велики борац за права жена. Била је оснивач *Удружења госпођа у Загребу* (1866), *Женске задруге у Новом Саду* (1867), *Више женске школе* у Панчеву (1870), *Више женске школе* у Новом Саду (1874), једна од оснивача и први председник (1903–1906) *Кола српских сестара*, приликом чијег оснивања је рекла да „сваки народ који тежи културном напретку мора увек да почне од детета, јер од начина образовања деце зависи будућност народа из кога су поникли". Савка се с ентузијазмом бавила и афирмацијом народне радиности и рукотворина српских жена на домаћим и страним изложбама, а пиротски ћилим називала је „поезијом женске руке који је у равни са српским народним песмама". Била је почасни председник више женских удружења, „мајка свога народа", како ју је назвао Алекса Шантић, и једна од значајних бораца за права жена у Аустроугарској и Европи.

Отац Војислава Субботића био је Јован Субботић, од оца Јоакима (Аћима) Субботића, свештеника у Добринцима у Срему, и мајке Саре. Јован је докторирао филозофију (1936) и права (1838) на Универзитету у Пешти. Радио је као адвокат, био је песник и драмски писац, један од предводника српског народа у Војводини, уредник *Летописа Матице српске* (1842–1847), човек који је сазвао и руководио Скупштином Срба у Пешти (1848) да би се пред предстојеће бурне догађаје дефинисали захтеви српског народа у Аустроугарској, учесник Мајске скупштине у Сремским Карловцима (1848), на којој је, између осталог, основана Карловачка патријаршија и за патријарха изабран Јосиф Рајачић. Био је изасланик Срба у Угарском сабору, у коме је износио идеје блиске идејама Светозара Милетића, и у Хрватском сабору, у коме је 1867. поднео предлог о равноправности српског имена и ћирилице, био управник *Земаљског казалишта* у Загребу, у Новом Саду председник *Матице српске* и челник *Друштва за Српско народно позориште*, чији задатак је био да води бригу око финансирања Позоришта (које је основано 16/28. јула 1861. године) и уредник листа *Народ*. Радио је на повезивању српских и хрватских политичких центара, Новог Сада, Загреба, Осијека и Београда итд.

Јован и Савка Субботић су имали шесторо деце: Виду, Верицу, Бранисава, Озрена,

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Дејана и Војислава. Вида и Озрен су рано умрли од малих богиња. Бранислав је био дипломата, Дејан (Деан) је завршио руске војне школе и у чину генерал-лајтнанта био је губернатор Амурских провинција (Манџурије) у Русији. Као добровољац дошао је у Србију са генералом Черњајевим и борио се у Првом српско-турском рату 1876/7, а затим је био почасни српски генерални конзул на Криму. Био је учесник оба балканска и Првог светског рата. Умро је 1920. године. Сахрањен је у породичној гробници на земунском гробљу.

Животна и професионална биографија Војислава Ј. Субботића

Војислав Субботић се родио у Новом Саду. Основну школу учио је у Загребу, а гимназију у Новом Саду. Медицину је студирао најпре у Бечу, затим у Паризу, па поново у Бечу. Током студија био је демонстратор код Карла Рокитанског, славног бечког професора патологије. Студије медицине завршио је у Бечу 1881. године, у 22. години живота.

Хирургију је специјализирао код славног професора **Едуарда Алберта** (1841–1900), шефа *Прве универзитетске хируршке клинике* у Бечу, хирурга који се снажно залагао за увођење антисепсе, који је увео бројне нове оперативне захвате у хирургију, јуностомију, нефректомију, серо-серозни шав црева („шав по Алберту“), први извео трансплантацију живца и др., поставио индикације за многе оперативне захвате и написао више уџбеника. Учио је и код **Карела Мајдла** (*Carel Maydl*), који је 1883. године у абдоминалну хирургију увео колостомију. Оба су потицали из Бохемије (Чешке). Субботић се са њима спријатељио, посебно са Мајдлом.

Године 1884. Субботић је постављен за физикуса и примарног лекара у Земуну, где је основао Хируршко одељење и постао управник Болнице. Већ у Земунској болници почео је да изводи сложене оперативне захвате и да публикује у водећим часописима у Аустроугарској и Немачкој.

Субботићу је нуђено место шефа Хируршког одељења у Загребу, али је он прихватио понуду, изгледа дату на предлог др Владана Ђорђевића, за место шефа Хируршког одељења Опште државне болнице (ОДБ) у Београду, које је преузео почетком 1889. године, и одмах почео велики посао на стварању и изграђивању српске хирургије. У разним публикацијама имену Војислава Ј. Субботића се додаје и реч „Старији“ да би се правила разлика од Војислава М. Суботића, првог српског неуропсихијатра, такође човека који је на много начина задужио српски народ, српску медицину, Српско лекарско друштво (СЛД) и Српско друштво Црвеног крста.

Др Војислав Субботић је у рутинску употребу увео општу и локалну анастезију. Изводио је од 300 до 400 операција годишње. Захваљујући вредноћи и одличном знању немачког, француског и мађарског језика, ажурно је пратио литературу и стално уводио нове, у литератури тек описане операције, које су развијане у водећим клиникама у Европи и Сједињеним Америчким Државама (САД), понекад их и модификујући, тако да је са успехом изводио практички све велике операције које су извођене на водећим клиникама у Европи тог времена. Др Леон Коен је с правом рекао да се на „хируршком одељењу г. Субботића ради по истим принципима као и на најнапреднијим клиникама“.

Свака таква операција била је својеврсни херојски подухват, јер у то време није било ендотрахеалне анестезије, парентералне рехидратације, трансфузије крви, антибиотика, и свега оног без чега се хирургија данас не може ни замислити. Неке сложене операције које је Субботић у тим условима са успехом изводио, после више од стотину година, успешно може да уради сразмерно мали број хирурга у нашој земљи.

Пошто је изводио операције на свим органима, данас практички нема ниједне гране хирургије у Србији за коју се не би могло рећи да је Субботић није засновао. Доказивање ове тврдње превазилази потребе овог пригодног текста, а заинтересовани читалац доказе може наћи у приложеној литератури. Нажалост, ове истине најчешће су потпуно непознате чак и нашим хирурзима „на гласу“, па нпр. као „прве“ операције у Србији понекада наводе операције које је Субботић са успехом изводио пуних 40 година раније. Док други народи љубоморно бележе најраније почетке своје хирургије и њених специјалистичких грана, због слабог познавања историје српске хирургије, наши, чак угледни професори хирургије, почетке „својих“ грана хирургије датирају и по више деценија након што су их Субботић и сар. рутински изводили.

Др Војислав Ј. Субботић је, упркос својој хируршкој величини, био скроман и, како је рекао др Михаило Петровић, оперисао је „при затвореним вратима, која су се отварала само лекарима, **јер ништа му мрскије није било но реклама новинарска коју је сматрао недостојном озбиљног лекара**“ (подвукао аутор), што би за многе данас требало да буде „лековито“.

Др Субботић је направио скице према којима је једна пројектантска кућа у Будимпешти израдила пројекте по којима су 1907. године завршили „Хируршки павиљони“ на Западном Врачару, у то време међу модернијим у Европи, који су служили до седамдесетих година XX века, када су порушени ради изградње Клиничког центра Медицинског факултета.

Др Војислав Ј. Субботић је био један од најбољих „активиста“ СЛД-а у његовој преко 150 година дугој историји. Његов долазак у СЛД унео је сасвим нови дух и елан у рад. Само током прве године реферисао је око 60 пута, а стручни и научни ниво састанака СЛД-а подигнут је на европски ниво. Састанци Друштва постали су много занимљивији и едукативнији и знатно боље посећени. Уверен у потребу ужих специјализација у медицини и хирургији, Субботић је 22. и 23. децембра 1907. године организовао *Први састанак српских хирурга*, на коме је учествовало 65 учесника, од којих три из иностранства, и уредио је *Извештај о раду* (тј. Зборник), који је 1908. године на 94 стране штампан у Државној штампарији Краљевине Србије и у коме су *in extenso* штампана сва излагања и све дискусије и унете бројне илустрације. Крајем 1909. и почетком 1910. поред редовних састанака одржао је осам одвојених „састанака са лекарима који су се бавили оперативном медицином“. Као велики присталица зближавања јужнословенских хирурга, 5, 6, и 7. септембра. 1911. године организовао је *Први југословенски састанак за оперативну медицину* коме су присуствовала 132 хирурга из Србије, Бугарске и Аустроугарске, највише из њених покрајина а данашњих република, Словеније, Хрватске и Босне и Херцеговине, а било је и појединих учесника из Црне Горе, Турске и Чешке. Овај *састанак* је касније узет за *Први конгрес југословенских хирурга.* Субботић је са др Соломоном Ј. Алкалајом уредио зборник под називом *I југословенски састанак за оперативну медицину*, који је 1912. године на 538 страна штампала Нова штампарија Саве Раденковића и брата, у коме су такође *in extenso* штампани сви радови и све дискусије, укључујући 108 слика и једну табелу у боји (*II југословенски састанак за оперативну медицину* одржан је 5. и 6. септембра 1921. у организацији Субботићевог пријатеља, Мирослава пл. Чачковића, професора Медицинског факултета у Загребу).

Субботић је пет пута биран (укупно пет година) за председника СЛД-а, а за те године је др Букић Пијаде с пуним правом написао да су биле „**период научног полета**“.

Др Субботић је био велики српски патриота. Иако поданик Аустроугарске, он је два пута био добровољац у ратовима које је Србија водила; најпре је као 17-годишњак збрињавао рањенике на Дрини за време Првог српско-турског рата (1876/77), а затим као лекар и хирург рањенике у српско-бугарском рату (1985/86). У ратовима 1912. и 1913. године, као хирург и српски поданик, збрињавао је рањенике у Београду и Нишу, и одржао 13 састанака наших и страних хирурга који су притекли у помоћ српском војном санитету, на којима су анализирана ратна хируршка искуства и по потреби коригована хируршка доктрина у збрињавању ратних повреда. Нарочито се истиче нова доктрина збрињавања повреда већих крвних судова, артерија и вена њиховом реконструкцијом, уместо дотадашњег рутинског подвезивања (лигирања), што је била новина у светској хирургији, а што је 1913. године објављено у *Lancet*-у. У чланку у часопису *Surgery*, под насловом „The Matas/Soubbotitch connection“, славни амерички војни хирург, учесник Корејског и Вијетнамског рата Норман Рич (*Norman Rich*) и сар. написали су и следеће: „Soubbotitch /.../ initiated one of the first clinical programs that emphasized repair, rather than ligation, of injured arteries and veins. Surgeons from the capitals in Europe visited his clinic to assist in this effort, and the 1913 presentation in London included the experience of managing 77 injured large blood vessels, which resulted in 32 vascular repairs – 19 arteriorrhaphies and 13 venorrhaphies. **It is ironic that nearly 40 years passed before similar successful efforts were achieved during the latter part of the Korean Conflict (1952 to 1953)**“ (подвукао аутор овог чланка). Последња реченица у преводу гласи: „Иронија је да је прошло скоро 40 година пре него су слични успешни напори достигнути у другој етапи Корејског рата (1952. до 1953)“.

У Првом светском рату, након одлуке да се наша војска повуче преко Албаније, Врховна команда је била предвидела да др Субботић остане у Нишу и да се преда бугарском окупатору. Иако тешко болестан, он је то одбио и повукао се преко Албаније. Са Крфа је послат у Лондон и Париз, где је био изасланик Краљевине Србије у Међусавезничкој санитетској комисији. Године 1918. вратио се у Солун. Одбио је понуђено место хирурга у позадинској болници и отишао у Драгоманце, у легендарну Хируршку пољску болницу Друге армије војводе Степе Степановића, коју су основали и у њој радили његови ученици и сарадници, доктори Михаило Петровић, Леон Коен и Никола Крстић.

По повратку у Београд почео је обнову Хируршког одељења ОДБ, које је било опустошено и опљачкано.

Схватајући огроман значај Медицинског факултета на развој медицине у Србији, Субботић је био један од снажних заговорника његовог оснивања, које је иначе било предвиђено у *Закону о подизању Велике школе на ниво Универзитета*, усвојеном 1905. године. Како из разних разлога до оснивања Факултета није долазило, Субботић је као председник СЛД-а сазвао седницу Друштва 5. марта 1911. године, на коју је позвао и водеће политичке личности Србије, и у уводном излагању рекао: „Од вајкада је медицина важна грана културног живота народа, а центри у којима се она гаји и учи извори су уплива и утицаја, који допиру

често и до удаљених покрајина и народа. Питање о оснивању медицинског факултета на српском универзитету значајно је по народ и државу, не само стога што од његовог повољног решења зависи умногоме будућност народног здравља, медицинских знаности и медицинског сталежа у Србији, него и стога што је с њим тесно спојен правилни развитак српског универзитета и српске науке уопште".

Припреме за оснивање Факултета, у којима је уз Милана Јовановића Батута Субботић био најзначајнија личност, интензивиране су после балканских ратова. Коначно је у мају 1914. године било донето *Решење* о оснивању Медицинског факултета у Београду и почетку рада у јесен те године, које је омело избијање Првог светског рата. После његовог завршетка, Субботић је био један од оснивача Факултета. Указом регента Александра Карађорђевића, који је потписао и министар просвете Павле Маринковић, Субботић је постављен за првог редовног професора хирургије и изабран за првог продекана, а следеће, 1921. године, и за декана Медицинског факултета.

Кад су стасавали први студенти медицине којима је требало држати предавања из хирургије, Субботић се посебно спремао за тај велики дан који је толико дуго и тако жељно чекао. Нажалост, тај, један од највећих дана у његовом животу, није дочекао.

Умро је 4/17. XII 1923. године за столом на коме је било његово приступно предавање. Уместо њега, предавање је прочитао његов наследник на Катедри, ванредни професор Миливоје Костић.

Др Војислав J. Субботић је био члан бројних угледних страних стручних друштава: француског, немачког и међународног хируршког друштва, пештанског лекарског друштва, париске медицинске академије, Друштва ратних војних хирурга САД и Друштва ратних хирурга Енглеске. Био је и носилац бројних домаћих и страних одликовања и признања.

Субботић је стручне радове почео објављивати 1886. године. У Пештанском медицинском листу описао је актиномикозу, само девет година после првог описа болести од стране Долингера (*Dollinger*) (1877) и Израела (1888) (*Israel*). Године 1887. описао је „операциони случај цисте панкреаса" и био је четврти хирург у свету који је дијагностицирао и успешно оперисао овај хируршки ентитет. Године 1898. рецензирао је *Ратну санитетску службу* пуковника др Михаила Мике Марковића, начелника војног санитета Краљевине Србије, у којој је дао низ корисних савета и предлога. На *Првом конгресу српских лекара и природњака*, одржаном од 5. до 7. септембра 1904. године, држао је предавање „Прилог патологији апендицитиса". Објавио је неколико радова о цистама и другим обољењима слезине и описао повреду *ductus hepaticus*-а у току повреде трбуха. Писао је и о илеусу, улкусу, акутном и хроничном панкреатитису, туберкулози црева, хернијама и низу других хируршких болести, и удлаги за имобилизацију прелома дугих костију ноге, о епидемији пегавог тифуса у Србији и др. Већ је било речи о његовом најзначајнијем раду „Military experience of traumatic aneurisms", објављеном 1913. године у *Lancet*-у.

Потпуну Субботићеву библиографију вероватно није могуће саставити, али у списку који смо успели да направимо налази се најмање 38 радова, махом објављених у водећим страним часописима, најмање 31 приказ књига или чланака из стране литературе, више стотина приказа и дискусија забележених у записницима са састанака СЛД-а о оперисаним болесницима, датим на начин да је данас сваком иоле добро едукованом хирургу сасвим лако да их разуме. Његове, на састанцима СЛД-а забележене многобројне дискусије о другим, нехируршким болестима, говоре о његовом широком познавању целокупне медицине.

Одгојен у уређеној држави каква је била Аустроугарска, Субботић је још док је радио у Земуну сваке године подносио писмене *Извештаје о раду*. После преласка у Београд (1889) редовно је писао *Извештаје о раду Хируршког одељења Опште Државне Болнице*, које је слао Санитетском одељењу Министарства унутрашњих дела, који су делом сачувани у Архиву Србије, а од 1892. су скоро редовно штампани у *Српском архиву за целокупно лекарство*. Укупно је објавио 39 чињеничних *Извештаја* захваљујући којима је могуће са скоро потпуном сигурношћу реконструисати рад и развој хирургије на Хируршком одељењу ОДБ у Београду. Велика је штета што после његове смрти његов наследник није објавио ни један једини такав *Извештај*, што јако отежава изучавање развоја међуратне хирургије у Србији.

После свега, поставља се питање: Шта су послератне генерације урадиле да се одужимо овом великом сину српског народа? Исправан одговор би био: „Доста, али не све што би требало". Навешћемо најважније.

Улица у којој се налази деканат Медицинског факултета добила је име по Војиславу J. Субботићу. Бивша Друга хируршка клиника под руководством професора Војислава К. Стојановића је у амфитеатру Клинике поставила Субботићеву бисту, рад академика Николе (Коке) Јанковића. Професор Зоран Герзић је 1996. написао обиман прилог о Субботићу у првој књизи едиције Српске академије наука и уметности „Живот и дело српских научника". Аутор ових редова је 2003. године поводом 80. година од смрти професора Субботића у *Српском архиву за целокупно лекарство* објавио „Сећање" на Субботића. Хируршка секција СЛД-а (под његовим руководством) на 80. годишњицу смрти професора Субботића потпуно је обновила гробницу породице Субботић на земунском гробљу, која је била у врло лошем стању и на њој је поставила спомен-плочу, у холу зграде СЛД-а поставила је бронзану копију поменуте бисте, чије је копирање без накнаде одобрио аутор, академик Никола (Кока) Јанковић, који се постарао да буде савршено урађена, а у сали за састанке СЛД-а постављена је његова фотографија и уметничка слика куће у којој је становао док је живео и радио у Земуну. Уместо технички врло рђаве слике у свечаној сали Деканата Медицинског факултета, постављена је његова нова

према фотографији израђеној у Бечу, а на позадини је додата његова биографија. У неколико прилика одржана су предавања о оцу српске хирургије и, по нашем сазнању, начињена његова до сада најпотпунија библиографија.

Бројни знани и анонимни аутори су у разним публикацијама написали прилоге о Субботићу и његовом раду. Хемофарм из Вршца је финансирао израду постамента и бисте Војислава Субботића испред Деканата, рад прим. др Владимира Јокановића.

Сматрајући да све наведено ипак није довољно, Секција за историју медицине СЛД-а је 21. маја 2014. године упутила детаљно образложен предлог директорима Прве хируршке клинике и Клиничког центра Србије и министру здравља Републике Србије да се Првој хируршкој клиници да Субботићево име, како је то већ урађено за оснивача института за анатомију, хистологију, физиологију и судску медицину.

Ни од једне од наведених адреса није било одговора. Да ли је стогодишњица смрти Војислава Ј. Субботића прилика да се овај предлог реализује?

Судбина породице Војислава Ј. Субботића

Војислав Ј. Субботић је био ожењен Зором Марковић (1871–1927), са којом је имао сина Ивана (1893–1973), који је студирао права у Београду, Бечу, Паризу, а завршио у Лозани (1931), где је 1936. године одбранио и докторат. Радио је у југословенској дипломатској служби и успешно обавио читав низ деликантних дипломатских задатака, био делегат у *Друштву народа* и пред Други светски рат је био изасланик у Лондону. Сматра се да је његова најзначајнија улога била потписивање у име краљевине *Декларације у палати Saint James* 12. јуна 1941. године, која се сматра првим кораком у оснивању Уједињених нација. Указом од 4. августа 1941. стављен је на располагање министру спољних послова, а решењем Министарског савета Владе у избеглиштву од 12. августа 1941. постављен је за краљевског делегата при Црвеном крсту САД и да обавља још неке дужности. После рата остао је у САД, у Њујорку до 1948. године поново завршио права и до смрти 1973. године на Универзитету Колумбија у Њујорку био је професор права и доживотни потпредседник Адвокатске коморе САД и функционер низа других институција. Сахрањен је у породичној гробници на земунском гробљу.

Иван Субботић се 15. децембра 1933. године у посланству у Паризу оженио Анком Гођевац (1890–1983), која се као гимназијалка удала за др Драгољуба Бајаловића, са којим је имала ћерке Катарину (1910) и Ружу (1914). Он њега се 1921. године развела, завршила 8. разред гимназије, а затим наставила студије права у Бечу и Београду, у коме је дипломирала и докторирала и била први доктор правних наука на београдском Правном факултету. Бавила се међународним правом и писала дела из правних наука, бавила се књижевношћу, новинарством, публицистиком и била истакнути борац за права жена у Краљевини Југославији и Европи. Са супругом Иваном остала је у избеглиштву, сарађивала у избегличким часописима и била предавач на више америчких универзитета. И она је сахрањена у породичној гробници Субботићевих на земунском гробљу.

Са Анком Гођевац, која је у време склапања брака имала 43 године, Иван Субботић није имао деце, чиме се породица Војислава Ј. Субботића „угасила".

Захвалница

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Centenary of the death of Professor Vojislav J. Subbotić, father of Serbian surgery and the first full professor of surgery of the Faculty of Medicine in Belgrade (1859–1923)

Radoje Čolović

Serbian Academy of Sciences and Arts, Belgrade, Serbia

SUMMARY

These days (December 4, 1923 according to the Julian calendar, or December 17, 2023, according to the Gregorian calendar) we mark the centenary of the death of the father of Serbian surgery, Professor Vojislav Subbotić, one of the two main founders of the Faculty of Medicine in Belgrade in 1920, one of the first (full) professors of the Faculty, the first professor of surgery, the first vice dean and the second dean, the first surgeon elected dean, founder and chair of the Surgery Department, founder and first head of the University Surgical Clinic, who held office of president of the Serbian Medical Society for a term of five years, who organized the "First Meeting of Serbian Surgeons" (1907) and the "First Yugoslavian meeting of operative medicine" (1911), the most versatile and most widely acclaimed Serbian surgeon of all time, whose pioneer achievements in vascular surgery remained unsurpassed for decades. The famous American surgeons N. M. Rich et al. wrote the following about his achievements in their article titled *The Matas/Soubotitch connection* published in the famous journal *Surgery* in 1983: "It is ironic that nearly 40 years passed before similar successful efforts were achieved during the latter part of the Korean Conflict (1952 to 1953)".

Keywords: Vojislav J. Subbotić; founder of all branches of surgery; Serbia

Пре подношења рукописа Уредништву часописа „Српски архив за целокупно лекарство“ (СА) сви аутори треба да прочитају Упутство за ауторе (*Instructions for Authors*), где ће пронаћи све потребне информације о писању и припреми рада у складу са стандардима часописа. Веома је важно да аутори припреме рад према датим пропозицијама, јер уколико рукопис не буде усклађен с овим захтевима, Уредништво ће одложити или одбити његово публиковање. Радови објављени у СА се не хонорарашу. За чланке који ће се објавити у СА, самом понудом рада Српском архиву сви аутори рада преносе своја ауторска права на издавача часописа – Српско лекарско друштво.

ОПШТА УПУТСТВА. СА објављује радове који до сада нису нигде објављени, у целости или делом, нити прихваћени за објављивање. СА објављује радове на енглеском и српском језику. Због боље доступности и веће цитираности препоручује се ауторима да радове свих облика предају на енглеском језику. У СА се објављују следеће категорије радова: уводници, оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови, актуелне теме, радови за праксу, радови из историје медицине и језика медицине, медицинске етике, регулаторних стандарда у медицини, извештаји са конгреса и научних скупова, лични ставови, наручени коментари, писма уреднику, прикази књига, стручне вести, *In memoriam* и други прилози. Оригинални радови, претходна и кратка саопштења, прикази болесника и случајева, видео-чланци, слике из клиничке медицине, прегледни радови и актуелне теме, публикују се искључиво на енглеском језику, а остале врсте радова се могу публиковати и на српском језику само по одлуци Уредништва. Радови се увек достављају са сажетком на енглеском и српском језику (у склопу самог рукописа). Текст рада куцати у програму за обраду текста *Word*, фонтом *Times New Roman* и величином слова 12 тачака (12 *pt*). Све четири маргине подесити на 25 *mm*, величину странице на формат А4, а текст куцати с двоструким проредом, левим поравнањем и увлачењем сваког пасуса за 10 *mm*, без дељења речи (хифенације). Не користити табулаторе и узастопне празне карактере (спејсове) ради поравнања текста, већ алатке за контролу поравнања на лењиру и *Toolbars*. За прелазак на нову страну документа не користити низ „ентера“, већ искључиво опцију *Page Break*. После сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*. Подаци о коришћеној литератури у тексту означавају се арапским бројевима у угластим заградама – нпр. [1, 2], и то редоследом којим се појављују у тексту. Странице нумерисати редом у доњем десном углу, почев од насловне стране.

При писању текста на енглеском језику треба се придржавати језичког стандарда *American English* и користити кратке и јасне реченице. За називе лекова користити искључиво генеричка имена. Уређаји (апарати) се означавају фабричким називима, а име и место произвођача треба навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или супскрипту (нпр. ^{99}Tc, *IL*-6, O_2, $Б_{12}$, *CD*8). Уколико се нешто уобичајено пише курзивом (*italic*), тако се и наводи, нпр. гени (*BRCA1*).

Уколико је рад део магистарске тезе, односно докторске дисертације, или је урађен у оквиру научног пројекта, то треба посебно назначити у Напомени на крају текста. Такође, уколико је рад претходно саопштен на неком стручном састанку, навести званичан назив скупа, место и време одржавања, да ли је рад и како публикован (нпр. исти или другачији наслов или сажетак).

КЛИНИЧКА ИСТРАЖИВАЊА. Клиничка истраживања се дефинишу као истраживања утицаја једног или више средстава или мера на исход здравља. Регистарски број истраживања се наводи у последњем реду сажетка.

ЕТИЧКА САГЛАСНОСТ. Рукописи о истраживањима на људима треба да садрже изјаву у виду писаног пристанка испитиваних особа у складу с Хелсиншком декларацијом и одобрење надлежног етичког одбора да се истраживање може извести и да је оно у складу с правним стандардима. Експериментална истраживања на хуманом материјалу и испитивања вршена на животињама треба да садрже изјаву етичког одбора установе и треба да су у сагласности с правним стандардима.

ИЗЈАВА О СУКОБУ ИНТЕРЕСА. Уз рукопис се прилаже потписана изјава у оквиру обрасца *Submission Letter* којом се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. За додатне информације о различитим врстама сукоба интереса посетити интернет-страницу Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME; http://www.wame.org*) под називом „Политика изјаве о сукобу интереса“.

АУТОРСТВО. Све особе које су наведене као аутори рада треба да се квалификују за ауторство. Сваки аутор треба да је учествовао довољно у раду на рукопису како би могао да преузме одговорност за целокупан текст и резултате изнесене у раду. Ауторство се заснива само на: битном доприносу концепцији рада, добијању резултата или анализи и тумачењу резултата; планирању рукописа или његовој критичкој ревизији од знатног интелектуалног значаја; завршном дотеривању верзије рукописа који се припрема за штампање.

Аутори треба да приложе опис доприноса појединачно за сваког коаутора у оквиру обрасца *Submission Letter*. Финансирање, сакупљање података или генерално надгледање истраживачке групе сами по себи не могу

оправдати ауторство. Сви други који су допринели изради рада, а који нису аутори рукописа, требало би да буду наведени у Захвалници с описом њиховог доприноса раду, наравно, уз писани пристанак.

ПЛАГИЈАРИЗАМ. Од 1. јануара 2019. године сви рукописи подвргавају се провери на плагијаризам/аутоплагијаризам преко *SCIndeks Assistant* – Cross Check (iThenticate). Радови код којих се докаже плагијаризам/аутоплагијаризам биће одбијени, а аутори санкционисани.

НАСЛОВНА СТРАНА. На првој страници рукописа треба навести следеће: наслов рада без скраћеница; предлог кратког наслова рада, пуна имена и презимена аутора (без титула) индексирана бројевима; званичан назив установа у којима аутори раде, место и државу (редоследом који одговара индексираним бројевима аутора); на дну странице навести име и презиме, адресу за контакт, број телефона, факса и имејл адресу аутора задуженог за кореспонденцију.

САЖЕТАК. Уз оригинални рад, претходно и кратко саопштење, преглед литературе, приказ случаја (болесника), рад из историје медицине, актуелну тему, рад за рубрику језик медицине и рад за праксу, на другој по реду страници документа треба приложити сажетак рада обима 100–250 речи. За оригиналне радове, претходно и кратко саопштење сажетак треба да има следећу структуру: Увод/Циљ рада, Методе рада, Резултати, Закључак; сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) статистичке анализе и ниво значајности. Закључак не сме бити уопштен, већ мора бити директно повезан са резултатима рада. За приказе болесника сажетак треба да има следеће делове: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак; сегменте такође писати као посебан пасус који почиње болдованом речи. За остале типове радова сажетак нема посебну структуру.

КЉУЧНЕ РЕЧИ. Испод Сажетка навести од три до шест кључних речи или израза. Не треба да се понављају речи из наслова, а кључне речи треба да буду релевантне или описне. У избору кључних речи користити *Medical Subject Headings – MeSH (http://www.nlm.nih.gov/mesh).*

ПРЕВОД НА СРПСКИ ЈЕЗИК. На трећој по реду страници документа приложити наслов рада на српском језику, пуна имена и презимена аутора (без титула) индексирана бројевима, званичан назив установа у којима аутори раде, место и државу. На следећој – четвртој по реду – страници документа приложити сажетак (100–250 речи) с кључним речима (3–6), и то за радове у којима је обавезан сажетак на енглеском језику. Превод појмова из стране литературе треба да буде у духу српског језика. Све стране речи или синтагме за које постоји одговарајуће име у нашем језику заменити тим називом. Уколико је рад у целости на српском језику, потребно је превести називе прилога (табела, графикона, слика, схема) уколико их има, целокупни текст у њима и легенду на енглески језик.

СТРУКТУРА РАДА. Сви поднаслови се пишу великим масним словима (болд). Оригинални рад и претходно и кратко саопштење обавезно треба да имају следеће поднаслове: Увод (Циљ рада навести као последњи пасус Увода), Методе рада, Резултати, Дискусија, Закључак, Литература. Преглед литературе и актуелну тему чине: Увод, одговарајући поднаслови, Закључак, Литература. Првоименовани аутор прегледног рада мора да наведе бар пет аутоцитата (као аутор или коаутор) радова публикованих у часописима с рецензијом. Коаутори, уколико их има, морају да наведу бар један аутоцитат радова такође публикованих у часописима с рецензијом. Приказ случаја или болесника чине: Увод (Циљ рада навести као последњи пасус Увода), Приказ болесника, Дискусија, Литература. Не треба користити имена болесника, иницијале, нити бројеве историја болести, нарочито у илустрацијама. Прикази болесника не смеју имати више од пет аутора.

Прилоге (табеле, графиконе, слике итд.) поставити на крај рукописа, а у самом телу текста јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

СКРАЋЕНИЦЕ. Користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (стандардне скраћенице, као нпр. ДНК, сида, ХИВ, АТП). За сваку скраћеницу пун термин треба навести при првом навођењу у тексту, сем ако није стандардна јединица мере. Не користити скраћенице у наслову. Избегавати коришћење скраћеница у сажетку, али ако су неопходне, сваку скраћеницу објаснити при првом навођењу у тексту.

ДЕЦИМАЛНИ БРОЈЕВИ. У тексту рада на енглеском језику, у табелама, на графиконима и другим прилозима децималне бројеве писати са тачком (нпр. 12.5 ± 3.8), а у тексту на српском језику са зарезом (нпр. 12,5 ± 3,8). Кад год је то могуће, број заокружити на једну децималу.

ЈЕДИНИЦЕ МЕРА. Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – *m*, килограм (грам) – *kg* (*g*), литар – *l*) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), количину супстанце у молима (*mol*), а притисак крви у милиметрима живиног стуба (*mm Hg*). Све резултате хематолошких, клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (*SI*).

ОБИМ РАДОВА. Целокупни рукопис рада који чине – насловна страна, сажетак, текст рада, списак литературе, сви прилози, односно потписи за њих и легенда (табеле, слике, графикони, схеме, цртежи), насловна страна и сажетак на српском језику – мора износити за оригинални рад, рад из историје медицине и преглед литературе до 5000 речи, а за претходно и кратко саопштење, приказ болесника, актуелну тему, рад за праксу, едукативни чланак и рад за рубрику „Језик медицине“ до 3000 речи; радови за остале рубрике могу имати највише 1500 речи.
Видео-радови могу трајати 5–7 минута и бити у формату *avi, mp4*(*flv*). У првом кадру филма мора се навести: у наднаслову Српски архив за целокупно лекарство, наслов рада, презимена и иницијали имена и средњег слова свих аутора рада (не филма), година израде. У другом кадру мора бити уснимљен текст рада у виду апстракта до 350 речи. У последњем кадру филма могу се навести имена техничког особља (режија, сниматељ, светло, тон, фотографија и сл.). Уз видео-радове доставити: посебно текст у виду апстракта (до 350 речи), једну фотографију као илустрацију приказа, изјаву потписану од свег техничког особља да се одричу ауторских права у корист аутора рада.

ПРИЛОЗИ РАДУ су табеле, слике (фотографије, цртежи, схеме, графикони) и видео-прилози.

Свака табела треба да буде сама по себи лако разумљива. Наслов треба откуцати изнад табеле, а објашњења испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле цртати искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће чинити мрежу табеле. Десним кликом на мишу – помоћу опција *Merge Cells* и *Split Cells* – спајати, односно делити ћелије. Куцати фонтом *Times New Roman*, величином слова 12 *pt*, с једноструким проредом и без увлачења текста. Коришћене скраћенице у табели треба објаснити у легенди испод табеле. Уколико је рукопис на српском језику, приложити називе табела и легенду на оба језика. Такође, у једну табелу, у оквиру исте ћелије, унети и текст на српском и текст на енглеском језику (никако не правити две табеле са два језика!).

Слике су сви облици графичких прилога и као „слике“ у CA се објављују фотографије, цртежи, схеме и графикони. Слике означавају се арапским бројевима према редоследу навођења у тексту. Примају се искључиво дигиталне фотографије (црно-беле или у боји) резолуције најмање 300 *dpi* и формата записа *tiff* или *jpg* (мале, мутне и слике лошег квалитета неће се прихватати за штампање!). Уколико аутори не поседују или нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини. Уколико је рад неопходно илустровати са више слика, у раду ће их бити објављено неколико, а остале ће бити у е-верзији чланка као *PowerPoint* презентација (свака слика мора бити нумерисана и имати легенду).
Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видеоприказа у *e*-издању и објављена у штампаном издању. Уколико је рукопис на српском језику, приложити називе слика и легенду на оба језика.

Слике се у свесци могу штампати у боји, али додатне трошкове штампе сносе аутори.

Графикони треба да буду урађени и достављени у програму *Excel*, да би се виделе пратеће вредности распоређене по ћелијама. Исте графиконе прекопирати и у *Word*-ов документ, где се графикони означавају арапским бројевима према редоследу навођења у тексту. Сви подаци на графикону куцају се у фонту *Times New Roman*. Коришћене скраћенице на графикону треба објаснити у легенди испод графикона. У штампаној верзији чланка вероватније је да графикон неће бити штампан у боји, те је боље избегавати коришћење боја у графиконима, или их користити различитог интензитета. Уколико је рукопис на српском језику, приложити називе графикона и легенду на оба језика.

Цртежи и схеме се достављају у *jpg* или *tiff* формату. Схеме се могу цртати и у програму *CorelDraw* или *Adobe Illustrator* (програми за рад са векторима, кривама). Сви подаци на схеми куцају се у фонту *Times New Roman*, величина слова 10 *pt*. Коришћене скраћенице на схеми треба објаснити у легенди испод схеме. Уколико је рукопис на српском језику, приложити називе схема и легенду на оба језика.

ЗАХВАЛНИЦА. Навести све сараднике који су допринели стварању рада а не испуњавају мерила за ауторство, као што су особе које обезбеђују техничку помоћ, помоћ у писању рада или руководе одељењем које обезбеђује општу подршку. Финансијска и материјална помоћ, у облику спонзорства, стипендија, поклона, опреме, лекова и друго, треба такође да буде наведена.

ЛИТЕРАТУРА. Списак референци је одговорност аутора, а цитирани чланци треба да буду лако приступачни читаоцима часописа. Стога уз сваку референцу обавезно треба навести *DOI* број чланка (јединствену ниску карактера која му је додељена) и *PMID* број уколико је чланак индексиран у бази *PubMed/MEDLINE*.

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту. Број референци не би требало да буде већи од 30, осим у прегледу литературе, у којем је дозвољено да их буде до 50, и у метаанализи, где их је дозвољено до 100. Број цитираних оригиналних радова мора бити најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Уколико се домаће монографске публикације и

чланци могу уврстити у референце, аутори су дужни да их цитирају. Већина цитираних научних чланака не би требало да буде старија од пет година. Није дозвољено цитирање апстраката. Уколико је битно коментарисати резултате који су публиковани само у виду апстракта, неопходно је то навести у самом тексту рада. Референце чланака који су прихваћени за штампу, али још нису објављени, треба означити са *in press* и приложити доказ о прихватању рада за објављивање.

Референце се цитирају према Ванкуверском стилу (униформисаним захтевима за рукописе који се предају биомедицинским часописима), који је успоставио Међународни комитет уредника медицинских часописа (*http://www.icmje.org*), чији формат користе *U.S. National Library of Medicine* и базе научних публикација. Примере навођења публикација (чланака, књига и других монографија, електронског, необјављеног и другог објављеног материјала) могу се пронаћи на интернет-страници *http://www.nlm.nih.gov/bsd/uniform_requirements.html*. Приликом навођења литературе веома је важно придржавати се поменутог стандарда, јер је то један од најбитнијих фактора за индексирање приликом класификације научних часописа.

ПРОПРАТНО ПИСМО (*SUBMISSION LETTER*). Уз рукопис обавезно приложити образац који су потписали сви аутори, а који садржи: 1) изјаву да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, 2) изјаву да су рукопис прочитали и одобрили сви аутори који испуњавају мерила ауторства, и 3) контакт податке свих аутора у раду (адресе, имејл адресе, телефоне итд.). Бланко образац треба преузети са интернет-странице часописа (*http://www.srpskiarhiv.rs*).

Такође је потребно доставити копије свих дозвола за: репродуковање претходно објављеног материјала, употребу илустрација и објављивање информација о познатим људима или именовање људи који су допринели изради рада.

ЧЛАНАРИНА, ПРЕТПЛАТА И НАКНАДА ЗА ОБРАДУ ЧЛАНКА. Да би рад био објављен у часопису *Српски архив за целокупно лекарство*, сви аутори који су лекари или стоматолози из Србије морају бити чланови Српског лекарског друштва (у складу са чланом 6. Статута Друштва) и измирити накнаду за обраду чланака (*Article Processing Charge*) у износу од 3000 динара. Аутори и коаутори из иностранства су у обавези да плате накнаду за обраду чланака (*Article Processing Charge*) у износу од 35 евра. Уплата у једној календарској години обухвата и све наредне, евентуалне чланке, послате на разматрање у тој години. Сви аутори који плате ову накнаду могу, уколико то желе, да примају штампано издање часописа. Треба напоменути да ова уплата није гаранција да ће рад бити прихваћен и објављен у *Српском архиву за целокупно лекарство*. Обавеза плаћања накнаде за обраду чланка не односи се на студенте основних студија и на претплатнике на часопис.

Установе (правна лица) не могу преко своје претплате да испуне овај услов аутора (физичког лица). Уз рукопис рада треба доставити копије уплатница за чланарину и претплату / накнаду за обраду чланка, као доказ о уплатама, уколико издавач нема евиденцију о томе.
Часопис прихвата донације од спонзора који сносе део трошкова или трошкове у целини оних аутора који нису у могућности да измире накнаду за обраду чланка (у таквим случајевима потребно је часопису ставити на увид оправданост таквог спонзорства).

СЛАЊЕ РУКОПИСА. Рукопис рада и сви прилози уз рад достављају се искључиво електронски преко система за пријављивање на интернет-страници часописа: *http://www.srpskiarhiv.rs*

НАПОМЕНА. Рад који не испуњава услове овог упутства не може бити упућен на рецензију и биће враћен ауторима да га допуне и исправе. Придржавањем упутства за припрему рада знатно ће се скратити време целокупног процеса до објављивања рада у часопису, што ће позитивно утицати на квалитет чланака и редовност излажења часописа.

За све додатне информације, молимо да се обратите на доле наведене адресе и број телефона.

АДРЕСА:
Српско лекарско друштво
Уредништво часописа „Српски архив за целокупно лекарство“
Ул. краљице Наталије 1
11000 Београд
Србија

Телефони: (+381 11) 409-2776, 409-4479
E-mail: *office@srpskiarhiv.rs*
Интернет адреса: *http://www.srpskiarhiv.rs*

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The papers are always submitted with Summary in both English and Serbian, included in the manuscript file. The text of the manuscript should be typed in *MS Word* using the *Times New Roman* typeface, and font size 12 pt. The text should be prepared with margins set to 25 mm and onto A4 paper size, with double line spacing, aligned left and the initial lines of all paragraphs indented 10 mm, without hyphenation. Tabs and successive blank spaces are not to be used for text alignment; instead, ruler alignment control tool and *Toolbars* are suggested. In order to start a new page within the document, *Page Break* option should be used instead of consecutive enters. Only one space follows after any punctuation mark. If special signs (symbols) are used in the text, use the *Symbol* font. References cited in the text are numbered with Arabic numerals within parenthesis (for example: [1, 2]), in order of appearance in the text. Pages are numbered consecutively in the right bottom corner, beginning from the title page.

When writing text in English, linguistic standard American English should be observed. Write short and clear sentences. Generic names should be exclusively used for the names of drugs. Devices (apparatuses, instruments) are termed by trade names, while their name and place of production should be indicated in the brackets. If a letter-number combination is used, the number should be precisely designated in superscript or subscript (i.e., 99Tc, IL-6, O2, B12, CD8). If something is commonly written in italics, such as genes (e.g. BRCA1), it should be written in this manner in the paper as well.

If a paper is a part of a master's or doctoral thesis, or a research project, that should be designated in a separate note at the end of the text. Also, if the article was previously presented at any scientific meeting, the name, venue and time of the meeting should be stated, as well as the manner in which the paper had been published (e.g. changed title or abstract).

CLINICAL TRIALS. Clinical trial is defined as any research related to one or more health related interventions in order to evaluate the effects on health outcomes. The trial registration number should be included as the last line of the Summary.

ETHICAL APPROVAL. Manuscripts with human medical research should contain a statement that the subjects' written consent was obtained, according to the Declaration of Helsinki, the study has been approved by competent ethics committee, and conforms to the legal standards. Experimental studies with human material and animal studies should contain statement of the institutional ethics committee and meet legal standards.

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AUTHORSHIP. All individuals listed as authors should be qualified for authorship. Every author should have participated sufficiently in writing the article in order to take responsibility for the whole article and results presented in the text. Authorship is based only on: crucial contribution to the article conception, obtaining of results or analysis and interpretation of results; design of manuscript or its critical review of significant intellectual value; final revision of the manuscript being prepared for publication.

The authors should enclose the description of contribution to the article of every co-author individually (within the Submission Letter). Funding, collection of data or general supervision of the research group alone cannot justify authorship. All other individuals having contributed to the preparation of the article should be mentioned in the *Acknowledgment* section, with description of their contribution to the paper, with their written consent.

PLAGIARISM. Since January 1, 2019 all manuscripts have been submitted via SCIndeks Assistant to Cross Check (software iThenticate) for plagiarism and auto-plagiarism control. The manuscripts with approved plagiarism/auto-plagiarism will be rejected and authors will not be welcome to publish in Serbian Achieves of Medicine.

TITLE PAGE. The first page of the manuscript (cover sheet) should include the following: title of the paper without any abbreviations; suggested running title; each author's full names and family names (no titles), indexed by numbers; official name, place and country of the institution in which authors work (in order corresponding to the indexed numbers of the authors); at the bottom of the page: name and family name, address, phone and fax number, and e-mail address of a corresponding author.

SUMMARY. Along with the original article, preliminary and short communication, review article, case report, article on history of medicine, current topic article, article for language of medicine and article for practitioners, the summary not exceeding 100–250 words should be typed on the second page of the manuscript. In original articles, the summary should have the following structure: Introduction/Objective, Methods, Results, Conclusion. Each segment should be typed in a separate paragraph using boldface. The most significant results (numerical values), statistical analysis and level of significance are to be included. The conclusion must not be generalized, it needs to point directly to the results of the study. In case reports, the summary should consist of the following: Introduction (final sentence is to state the objective), Case Outline (Outline of Cases), Conclusion. Each segment should be typed in a separate paragraph using boldface. In other types of papers, the summary has no special outline.

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TRANSLATION INTO SERBIAN. The third page of the manuscript should include: title of the paper in the Serbian language; each author's full name and family name (no titles), indexed by numbers; official name, place and country of the institution in which authors work. On the fourth page of the manuscript the summary (100–250 words) and keywords (3–6) should be typed, but this refers only to papers in which a summary and keywords are compulsory. The terms taken from foreign literature should be translated into comprehensible Serbian. All foreign words or syntagms that have a corresponding term in Serbian should be replaced by that term.

If an article is entirely in Serbian (e.g. article on history of medicine, article for "Language of medicine," etc.), captions and legends of all enclosures (tables, graphs, photographs, schemes) – if any – should be translated into English as well.

STRUCTURE OF THE MANUSCRIPT. All section headings should be in capital letters using boldface. Original articles and preliminary and short communications should have the following section headings: Introduction (objective is to be stated in the final paragraph of the Introduction), Methods, Results, Discussion, Conclusion, References. A review article and current topic include: Introduction, corresponding section headings, Conclusion, References. The firstly named author of a review article should cite at least five auto-citations (as the author or co-author of the paper) of papers published in peer-reviewed journals. Co-authors, if any, should cite at least one auto-citation of papers also published in peer-reviewed journals. A case report should consist of: Introduction (objective is to be stated in the final paragraph of the Introduction), Case Report, Discussion, References. No names of patients, initials or numbers of medical records, particularly in illustrations, should be mentioned. Case reports cannot have more than five authors. Letters to the editor need to refer to papers published in the *Serbian Archives of Medicine* within previous six months; their form is to be comment, critique, or stating own experiences. Publication of articles unrelated to previously published papers will be permitted only when the journal's Editorial Office finds it beneficial.

All enclosures (tables, graphs, photographs, etc.) should be placed at the end of the manuscript, while in the body of the text a particular enclosure should only be mentioned and its preferred place indicated. The final arrangement (position) of the enclosures will depend on page layout.

ABBREVIATIONS. To be used only if appropriate, for very long names of chemical compounds, or as well-known abbreviations (standard abbreviations such as DNA, AIDS, HIV, ATP, etc.). Full meaning of each abbreviation should be indicated when it is first mentioned in the text unless it is a standard unit of measure. No abbreviations are allowed in the title. Abbreviations in the summary should be avoided, but if they have to be used, each of them should be explained when first mentioned in the text of the paper.

DECIMAL NUMBERS. In papers written in English, including text of the manuscript and all enclosures, a decimal point should be used in decimal numbers (e.g. 12.5 ± 3.8), while in Serbian papers a decimal comma should be used (e.g. 12,5 ± 3,8). Wherever applicable, a number should be rounded up to one decimal place.

UNITS OF MEASURE. Length, height, weight and volume should be expressed in metric units (meter – m, kilogram – kg, gram – g, liter – l) or subunits. Temperature should be in Celsius degrees (°C), quantity of substance in moles (mol), and blood pressure in millimeters of mercury column (mm Hg). All results of hematological, clinical and biochemical measurements should be expressed in the metric system according to the International System of Units (SI units).

LENGTH OF PAPER. The entire text of the manuscript – title page, summary, the whole text, list of references, all

enclosures including captions and legends (tables, photographs, graphs, schemes, sketches), title page and summary in Serbian – must not exceed 5,000 words for original articles, review articles and articles on history of medicine, and 3,000 words for case reports, preliminary and short communications, current topics, articles for practitioners, educational articles and articles for "Language of medicine", congress and scientific meeting reports; for any other section maximum is 1,500 words.

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Serbian Archives of Medicine
Editorial Office
Kraljice Natalije 1
11000 Belgrade
Serbia
Phones: (+381 11) 409-2776, 409-4479
E-mail: *office@srpskiarhiv.rs*
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