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Kontakt adresa: Časopis Acta Medica Medianae, Medicinski fakultet, Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: acta@medfak.ni.ac.rs
Tel+381-18-4533001 lok. 122 fax. +381-18-4534336
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Autor slike na prednjoj stranici:
Ivan Mitić, sociolog i planinar

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NEUROLOGICAL DISORDERS IN PATIENTS WITH CORONAVIRUS DISEASE 2019

Sladjana Pavić[1], Željko Karganović[2,3], Aleksandra Pavić[4], Slobodan Jovićević[5]

In addition to respiratory symptoms, patients with coronavirus disease 2019 (COVID-19) often have neurological, cardiac, gastroenterological and other symptoms. The most common neurological disorders are headache, myalgia, dizziness, acute cerebrovascular disease, disorders of the senses of taste and smell. We examined clinical symptoms, comorbidities, demographic, hematological, and biochemical parameters of 230 patients who showed neurological symptoms during COVID-19. The diagnosis of COVID-19 was made by rapid antigen or PCR (polymerase chain reaction) test. The diagnosis of neurological disorders was made by neurological examination, computed tomography, electroencephalography, lumbar puncture. The severity of the disease was estimated based on the Australian guidelines for the clinical care of people with COVID-19. The Statistical Package for Social Sciences (SPSS, version 16) was used for statistical data analysis. The probability $P < 0.05$ was considered significant.

The most common age was 51-60 years (mean 52.7 ± 10.3). A significant majority of patients had fatigue/weakness (95.7%), fever (90.9%), cough (75.7%) and chest tightness/pain (65.2%). Comorbidities were present in 69.6% of respondents. The most common were cardiovascular diseases (90.6%) and obesity (82.5%). Other associated diseases were asthma/chronic obstructive pulmonary disease (50.6%), diabetes mellitus (40%), gastrointestinal (26.9%), psychiatric disorders (16.3%). The significant majority of patients had elevated levels of lactate dehydrogenase, creatine kinase and C-reactive protein (95.7%, 82.5% and 79.1%), as well as leukopenia (82.6%). Significant frequency of neurological symptoms included headache (94.3%), loss sense of smell/taste, myalgia (90.9%, 84.8%, 88.7%). Patients with severe disease were significantly more often older than 50 (78.2%), with comorbidities, dizziness and acute cerebrovascular disease.

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Key words: *COVID-19, severity of disease, neurological disorders*

[1]General Hospital Užice, Department of Infectious and Tropical Disease, Užice, Serbia
[2]General Hospital Užice, Department of Neurology, Užice, Serbia
[3]Academy of Applied Sciences Western Serbia, Užice Department, Užice, Serbia
[4]University of Belgrade, School of Medicine, Belgrade, Serbia
[5]General Hospital Užice, Emergency Services, Užice, Serbia

Contact: Slađana Pavić
17 M. Obrenovića St., 31000 Užice, Serbia
E-mail: sladjanapj@gmail.com

Introduction

On January 25, 2020, Chinese scientists announced the identification of a new, seventh member of the coronavirus family with the potential for human infection, soon to be called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (1). Infection with the new virus spread rapidly across the planet. The World Health Organization declared a pandemic of the SARS-CoV-2 virus on March 11, 2020 (2). Despite all the prevention and treatment measures taken, coronavirus disease 2019 (COVID-19) remains a major challenge for physicians and scientists worldwide (3). The number of patients and deaths is still high, despite the vaccine that is used in many countries.

The clinical picture of these patients usually includes general and respiratory symptoms: malaise, weakness, loss of appetite, fever, headache, cough, tightness and chest pain. In addition, patients with COVID-19 may have symptoms of damage to many other organ systems: cardiovascular, gastrointestinal, nervous, hematopoietic, immunological, urinary, reproductive, as well as behavioral changes (4). This is confirmed by pathological biopsy and autopsy findings proving the presence of SARS-CoV-2 virus not only in the lungs but also in other organs - spleen, liver, heart, kidneys and brain (5, 6).

Headache, dizziness, encephalopathy, acute cerebrovascular disease, sensory and taste disturbances, and neuralgia/mialgia are common neurological manifestations of COVID-19 (7). Virus neurotropism has been shown by its identification in brain tissue in 36.4% of autopsied fetuses (8). In addition, replication of the virus has been proven and its particles were detected in the structures and organs of the nervous system of infected persons (9).

The aim

The aim of the study was to analyze the neurological disorders in patients with COVID-19 and their frequency in relation to the severity of COVID-19.

Patients and methods

We examined a total of 230 patients with COVID-19 and neurological disorders who were treated at the Department of Infectious Diseases of the General Hospital Užice in the period from March 1, 2020 to September 1, 2021.

Patients with chronic neurological and malignant diseases were excluded from the study.

We analyzed demographic data (sex and age), subjective and objective symptoms (typical and neurological), presence of comorbidities, hematological and biochemical parameters: lactate dehydrogenase (LDH), creatine kinase (CK), C-reactive protein (C-RP).

The diagnosis of COVID-19 was made based on the detection of SARS-CoV-2 virus in the nasopharyngeal swab. Testing was performed in reference laboratories in the Republic of Serbia. A rapid immunochromatographic antigen test and a polymerase chain reaction (PCR) test were used. The tests were registered by the Agency for Medicines and Medical Devices of Serbia.

The diagnosis of acute cerebrovascular diseases (ACVD) was made by computed tomography (CT). All patients with epileptic seizures underwent electroencephalography and CT of the endocranium. Clinical methods - objective status, concentration and memory tests - were used to assess the existence of encephalopathy in our patients. For the purpose of differential diagnosis, computed tomography examinations and examination of cerebrospinal fluid were performed. Cerebrospinal fluid (CSF) was examined cytologically and biochemically. A culture of cerebrospinal fluid was performed. Hematological and biochemical analyses were performed by standard methods used in the Republic of Serbia. Pneumonia was confirmed by radiography or CT.

The severity of the disease was assessed according to Australian guidelines for the clinical care of people with COVID-19:

- Mild illness: no symptoms, or mild upper respiratory tract symptoms, or cough, new myalgia or asthenia without new shortness of breath or a reduction in oxygen saturation;

- Moderate illness: prostration, severe asthenia, fever > 38 ^{O}C or persistent cough, clinical or radiological signs of lung involvement, no clinical or laboratory indicators of clinical severity or respiratory impairment;

- Severe illness: respiratory rate ≥ 30 breaths/min, oxygen saturation ≤ 92% at a rest state arterial partial pressure of oxygen (PaO_2)/ inspired oxygen fraction (FiO_2) ≤ 300 (10).

Patients with critical illness were not included in the study.

All collected data were analyzed retrospectively.

The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL. USA) version 16.0 was used for statistical analysis with two-side tests and $P < 0.05$ was considered a significance difference.

Results

The investigated patients were aged from 19-85 years (52.7 ± 10.3). The most frequent age group included individuals in the sixth decade, from 50-60 years old.

The main characteristics of our patients with COVID-19 and neurological symptoms are presented in Table 1.

The clinical presentation was significantly dominated by fatigue/weakness, fever and cough in 220, 209 and 174 patients, respectively, as well as chest tightness/pain in 150 individuals.

The presence of comorbidities was statistically significant, most commonly manifested as cardiovascular disease (CVD) (145 patients), followed by obesity (132 patients), diabetes mellitus (DM) type II (91 patients), asthma/chronic obstructive pulmonary disease (COPD) (81 patients) and gastrointestinal disease (GID) (43 patients). The least patients (26) had psychiatric disorders (PD).

Biochemical and hematological findings showed high frequencies of patients for the following parameters: significantly elevated values of CK, LDH and C-RP (192, 220 and 182 patients, respectively) and decreases of leucocyte count (190 patients).

The most common neurological symptom in our patients with COVID-19 was headache (217 patients). A significant number of patients had hyposmia/anosmia and hypogeusia as well as myalgia in 199, 175 and 201 patients, respectively. Fifty-three patients had dizziness. ACVD was diagnosed in 25 patients, of which 16 (64%) patients had ischemic stroke, and 9 (36%) it was hemorrhage. The smallest number of respondents had encephalopathy and epilepsy (11 and 7 patients).

Proteinorachia was present in four patients with CST. Cytological examination of cerebrospinal fluid CSF did not show cells.

Neurological disorders of patients with COVID-19 are presented in Figure 1.

We further compared epidemiological and neurological parameters of patients with COVID-19 according to the severity of the disease.

We further compared epidemiological and neurological parameters of patients with COVID-19 according to the severity of the disease.

Significantly more patients with severe pneumonia were older than 50 years compared to those with mild disease and pneumonia (78.2% vs. 54.3%).

Table 1. Characteristics of total patients with COVID-19 with neurological symptoms

Characteristics		Nº (%)
Male gender		132 (57.4)
Years	19 - 30	4
	31 - 40	43 (18.7)
	41 - 50	48 (20.1)
	51 - 60	70 (30.4)
	61 - 70	36 (15.7)
	71 - 80	22 (9.6)
	> 80	7 (3.0)
Symptoms	Fatigue/weakness	220 (95.7)
	Body temperature>37^{0}C	209 (90.9)
	A cough	174 (75.7)
	Chest tightness/pain	150 (65.2)
	Diarrhea	93 (40.4)
	Nausea/vomiting	44 (19.1)
Comorbidities	CVD	145 (90.6)
	Obesity	132 (82.5)
	DM	91 (56.9)
	Asthma/COPD	81 (50.6)
	GID	43 (26.9)
	PD	26 (16.3)
	Total	160 (69.6)
Laboratory date	RBC < 4.1x10^{12} /L	78 (33.9)
	WBC < 4.5x10^9/L	190 (82.6)
	PLT < 150x10^9/L	137 (59.6)
	Hb < 12.5 g/dL	51 (22.2)
	CK > 198 U/L	192 (83.5)
	LDH > 241 U/L	220 (95.7)
	C-RP > 5 mg/L	182 (79.1)

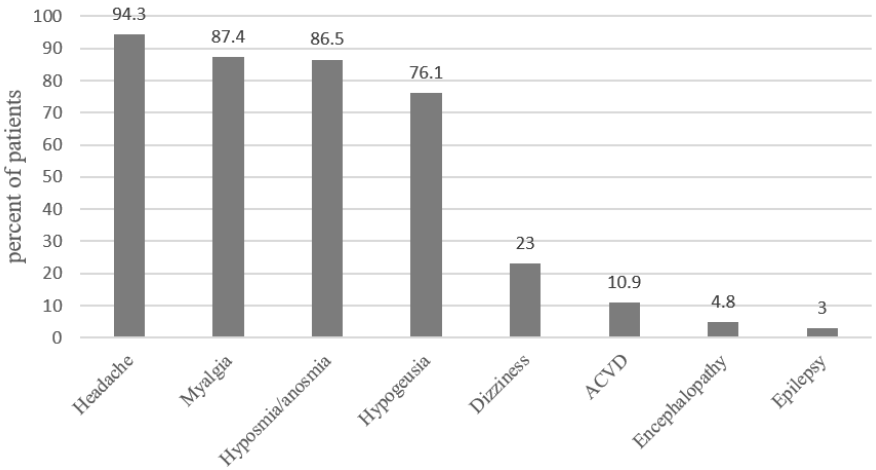

Figure 1. Neurological disorders of patients with COVID-19

The main comparative characteristics of our patients with COVID-19 in relation to the severity of the disease are presented in Table 2.

A cough and chest tightness/pain were significantly more common general symptoms in patients with a more severe clinical course of disease.

ACVD and dizziness were more common neurological disorders in patients with severe disease. The others neurological disorders (headache, encephalopathy, hyposmia, hypogeusia, myalgia) were not significantly different among the groups of respondents.

Comorbidities (obesity, DM, CVD, asthma /COPD, GID and PD) were significantly more frequent in patients with severe disease.

Thrombocytopenia was significantly more common in patients with severe clinical course.

Table 2. Comparative characteristics of the patients with COVID-19 and neurological disorders in relation to the severity of disease

Characteristics		Mild/moderate illness N° 138 (%)	Severe illness N° 92 (%)	P*
Gender	Male	85 (61.6)	47 (51.1)	0.345
	Female	53 (38.4)	45 (48.9)	0.261
Years	19 - 30	4	0	0.00
	31 - 40	36 (26.1)	7 (7.6)	
	41 - 50	35 (25.4)	13 (14.1)	
	51 - 60	47 (34.1)	23 (25.0)	
	61 - 70	9 (6.5)	27 (29.3)	
	71 - 80	5 (3.6)	17 (18.5)	
	> 80	2	5 (5.4)	
Symptoms	Fatigue/weakness	129 (93.4)	91 (98.9)	0.692
	Body temperature > 37 ^{0}C	119 (86.2)	90 (97.8)	0.393
	A cough	82 (59.4)	92(100)	0.001
	Chest tightness/pain	60 (43.5)	90 (97.8)	< 0.001
	Diarrhea	44 (31.9)	49 (53.2)	0.192
	Nausea/vomiting	20 (14.5)	24 (26.1)	0.069
Neurological disorders	Headache	129 (93.5)	88 (95.7)	0.872
	Dizziness	40 (29.0)	53 (57.6)	0.002
	Encephalopathy	4	7 (7.6)	ND
	ACVD	5 (3.6)	20 (21.7)	< 0.001
	Epilepsy	0	7 (7.6)	ND**
	Hyposmia	124 (89.9)	85 (92.4)	0.854
	Hypogeusia	115 (83.3)	80 (87.0)	0.777
	Myalgia	124 (89.9)	80 (87.0)	0.827
Comorbidities	Obesity	51 (37.0)	81 (88.0)	< 0.001
	DM	18 (13.0)	74 (80.4)	0.00
	CVD	60 (43.4)	85 (92.4)	< 0.001
	Asthma/COPD	20 (14.4)	61 (66.3)	< 0.001
	GID	12 (8.7)	31 (33.7)	< 0.001
	PD	9 (6.5)	17 (18.5)	0.016
Laboratory date	RBC < 4.1x10^{12} /L	40 (51.2)	38 (48.8)	0.809
	WBC < 4.5x10^9/L	78 (41.1)	52 (56.5)	0.119
	PLT < 150x10^9/L	47 (34.1)	90 (97.8)	< 0.001
	Hb < 12.5 g/dL	28 (20.3)	23 (25)	0.485
	CK > 198 U/L	106 (76.8)	86 (93.5)	0.201
	LDH > 241 U/L	104 (75.4)	92 (100)	0.063
	C-RP > 5 mg/L	83 (60.1)	71 (77.1)	0.381

*Statistical analysis performed in five or more patients
** ND, not determined

Discussion

Corona viruses are not primarily neurotropic viruses and their primary target is respiratory epithelium. The target receptor for attachment to cell and subsequent internalization is through the angiotensin converting enzyme-2 receptor (ACE 2). After entry into the cell, the virus RNA is released in the cytoplasm subsequently translated and replicated, after formation of envelope protein and incorporation of RNA into it, the virus is released in the circulation (11).

Epithelial cell damage causes general and respiratory symptoms that were the most common in our patients, in accordance with the already described symptoms of COVID-19 (7, 12). These symptoms are expected to be more common in severe disease. It is also expected that people with comorbidities have a more severe clinical course of the disease.

A significant number of our patients experienced chest tightness/pain. Pulmonary angiography did not indicate pulmonary thromboembolism in these patients. In the vast majority, the pain was short-lived. Often, the pain stopped after admission to the hospital and the application of general therapy. Oliviero et al. have shown that chest pain may be a symptom of increased anxiety present in COVID-19 (13). The same authors describe the occurrence of more frequent gastrointestinal symptoms with increased anxiety (13). Gastrointestinal symptoms were also present in our patients and were probably not just a consequence of the effects of SARS-CoV-2. We cannot draw a reliable conclusion as we did not measure the degree of anxiety of our patients.

A small proportion of people with COVID-19 can experience significant chest pains, which are mostly brought on by breathing deeply, coughing or sneezing. This is likely to be caused by the virus directly affecting their muscles, lungs and peripheral nerve (11).

Myalgia was present in the vast majority of our patients, with no significant difference in the severity of the clinical course. It was accompanied by elevated CK and LDH values. This is expected in relation to other research (7). Mao et al. concluded that it was not clear whether this was due to the direct effect of virus on muscle tissue. The other possible mechanism proposed was the infection-mediated immune response that causing elevated pro-inflammatory cytokines in serum resulting in skeletal muscle damage (7). Lactate levels increased due to surplus cell damage during COVID-19 (14). Lactate and H^+ ion accumulation occur in the cytosol, and the cytosol pH decreases (15). ATP synthesis is reduced due to anaerobic glycolysis. Decreased ATP (adenosine triphosphate) synthesis and low intracellular pH cause pain and fatigue (16, 17). During hypoxic ischemia, the increase of growth factors, cytokines levels, ischemic conditions, and microvascular changes can trigger pain by overexpression in the dorsal root ganglion (18).

Although COVID-19 preferentially affects the respiratory and cardiovascular system, up to 84% of patients show neurological symptoms (19).

ACE 2 receptors are also found in glial cells in brain and spinal neurons. During the viremia phase of illness, disruption of blood brain barrier causes the virus to enter the brain directly. Another postulated mechanism is the invasion of peripheral nerve terminals by CoV which then gains entry to the CNS through the synapse connected route (11).

Headache, dizziness, taste and smell dysfunctions were the most frequently reported neurological symptoms in COVID-19, as well as ours (7, 20). In patients with COVID-19 and available data on the severity of disease, headache was reported more frequently in mild or moderate compared to severe or critical disease (20). We do not confirm this conclusion, but our study did not include patients with critical illness. We observed more frequent dizziness in the more severe clinical course of the disease. We also observed a higher incidence of ACVD in patients with severe disease. This can be explained by the conclusion reached by Tehrani et al. that dizziness is more common in people with ACVD (21).

The neuropathogenic effect of SARS-CoV-2 is likely to be achieved by both hypoxic brain damage and immune mediated damage. Severe pneumonia was followed by peripheral vasodilatation, hypercarbia, hypoxia and anaerobic metabolism with accumulation of toxic compounds. These can result in neuronal swelling and brain edema which results in neurological damage (22). Immune mediated injury is mainly due to the cytokine storms with increased levels of inflammatory cytokines and activation of T lymphocytes, macrophages, and endothelial cells (23). Immune-mediated mechanisms of ACVD with consequent dizziness are described (24).

Cerebrovascular disease has been associated with an increased disease severity in patients with COVID-19 (7, 25). We noticed this in our research as well.

Since SARS-CoV-2 binds to ACE2, some patients with underlying hypertension may have unusually high blood pressure and increased risk of intracranial hemorrhage after SARS-CoV-2 infection. Severely low platelets are also an important manifestation of critical SARS-CoV-2 infection, as well as an independent risk factor for acute cerebrovascular events (5).

Our patients also had the progression of thrombocytopenia with the progression of the clinical course. In addition, the most common comorbidity in our patients was CVD. Chinese researchers have also identified CVD as the most common morbidity (7). Their research confirms older age as a risk factor for the progression of the clinical course of the disease, as well as ours.

Some authors describe that the cause of ACVD is more often ischemia than hemorrhage which was confirmed by our examination (26).

This is supported by the conclusions that the hypercoagulability seen in patients with COVID-19 may predispose to a stroke while disseminated intravascular coagulation is more commonly associated with the disease progression (27, 28).

The smell and taste dysfunction was present in a significant majority of our patients. The result corresponds to the order of other authors (29).

Some authors have described a lower incidence of sense of smell loss. Our study included anosmia and hyposmia in a significantly larger sample of patients compared to the mentioned study (7, 30). Mao and Lechien have described higher incidence of taste and smell disorders in mild/moderate clinical forms compared to severe/critical patients (7, 31). In a large Iranian study, taste loss was significantly more common than a loss of smell (32). In our study, no significant difference in the loss of the sense of taste and smell was noticed either in the type of senses or in the severity of the clinical appearance.

Variations among populations infected with different virus mutations were considered. It has been observed that populations infected predominantly with the G614 virus had a much higher prevalence of anosmia compared with the same ethnic populations infected mostly with the D614 virus strain (33).

A smaller number of our respondents had encephalopathy compared to the results of Chinese researchers (34). Encephalopathy can be caused by hypoxia, especially present in patients with asthma/ COPD.

CSF culture ruled out the presence of bacterial diseases. We were not able to diagnose SARS-CoV-2 in CSF. CSF results of our four patients revealed elevated proteins, without pleocytosis. Cases of SARS-CoV-2 in CSF with and without pleocytosis have been reported in the literature (35, 36). It has already been noticed that viral meningoencephalitis may occur frequently in the lack of CSF pleocytosis (37). Proteinorachia in COVID-19 has been described by other authors (38).

Only 3% of our patients had EPI. It is similarly described in the literature with the hypothesis that SARS-CoV-2 could trigger seizures through a neurotropic pathogenic mechanism (39).

Conclusion

SARS-CoV-2 most commonly causes respiratory symptoms, but may infect nervous system.

The most common neurological symptoms are headache, disorders of the senses of taste and smell, and myalgia. Most patients with neurological problems have comorbidities, most commonly cardiovascular disease and obesity. Patients with severe COVID-19 have significantly more neurological manifestations in terms of acute cerebrovascular disease and dizziness.

Careful and timely examination of patients with COVID-19 and neurological symptoms is necessary to avoid delayed diagnosis or misdiagnosis. A multidisciplinary team is needed to carefully monitor multiorgan functions.

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NEUROLOŠKI POREMEĆAJI KOD BOLESNIKA SA KORONAVIRUSNOM BOLEŠĆU 2019

Slađana Pavić[1], Željko Karganović[2,3], Aleksandra Pavić[4], Slobodan Jovićević[5]

[1]Opšta bolnica Užice, Odeljenje za infektivne i tropske bolesti, Užice, Srbija
[2]Opšta bolnica Užice, Odeljenje za neurologiju, Užice, Srbija
[3]Akademija strukovnih studija Zapadna Srbija, Odeljenje Užice, Užice, Srbija
[4]Univerzitet u Beogradu, Medicinski fakultet, Beograd, Srbija
[5]Opšta bolnica Užice, Odeljenje urgentne medicine, Užice, Srbija

Kontakt: Slađana Pavić
M. Obrenovića 17, 31000 Užice, Srbija
E-mail: sladjanapj@gmail.com

Pored respiratornih simptoma, bolesnici sa bolešću izazvanom korona virusom COVID-19 često imaju neurološke, kardiološke, gastroenterološke i druge simptome. Najčešće neurološke tegobe su glavobolja, mijalgije, vrtoglavica, pojava akutne cerebrovaskularne bolesti, kao i poremećaji čula ukusa i mirisa. Retrogradno, analizirali smo klinički tok, komorbiditete, demografske, hematološke i biohemijske karakteristike 230 bolesnika, koji su u kliničkoj slici COVID-19 virusa ispoljili neurološke simptome. Dijagnoza oboljenja izazvanog virusom COVID-19 postavljena je na osnovu brzog antigenskog i PCR (polimeraza lančane reakcije) testa. U dijagnostici neuroloških poremećaja, osim neurološkog pregleda, primenjivane su kompjuterizovana tomografija, elektroencefalografija, kao i lumbalna punkcija. Težina bolesti procenjena je na osnovu Australijskog vodiča za težinu kliničke slike oboljenja izazvanog virusom COVID-19. Statistička analiza rađena je pomoću Statističkog paketa za društvene nauke (SPSS, verzija 16). Verovatnoća $p < 0,05$ smatrana je značajnom.

Najčešći uzrast ispitanika bio je od 51 godine do 60 godina (prosek 52,7 godina ± 10,3 godine). Značajna većina bolesnika osećala je slabost/malaksalost (95,7%), imala je povišenu telesnu temperaturu (90,9%), kašalj (75,7%) i osećala je stezanje/bol u grudima (65,2%). Komorbiditeti su bili prisutni kod 69,6% ispitanika. Najčešće su bile kardiovaskularne bolesti (90,6%) i gojaznost (82,5%), zatim astma / hronična opstruktivna bolest pluća (50,6%), diabetes mellitus (40%), gastrointestinalne (26,9%) i psihijatrijske bolesti (16,3%). Značajna većina bolesnika imala je u laboratorijskim analizama povišen nivo laktat dehidrogenaze, kreatin kinaze, c-reaktivnog proteina (95,7%; 82,5% i 79,1%) i leukopeniju (82,6%). Značajna učestalost neuroloških simptoma podrazumevala je glavobolju (94,3%), poremećaj čula mirisa i ukusa i mijalgije (90,9%; 84,8%; 88,7%). U odnosu na težinu oboljenja izazvanog COVID-19 virusom, bolesnici sa težom bolešću bili su značajno češće uzrasta preko 50 godina (78,2%), sa prisustvom vrtoglavice i akutne cerebrovaskularne bolesti. Ovi bolesnici značajno češće imali su komorbiditete.

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THREE-YEAR COMPARATIVE ANALYSIS OF THE PRESENCE OF MENINGIOMAS AND SECONDARY DEPOSITS IN THE BRAIN

Vesna Nikolov[1,2], Boban Jelenković[1,2], Miša Radisavljević[1,2], Nikola Stojanović[2]

About 16% of the world's population is covered by registration systems that provide cancer incidence statistics, while mortality data are available for about 29%. The percentage of metastatic changes in the brain, as secondary deposits, is also increasing. Meningiomas are most often slow-growing, benign, primary intracranial tumors in adults, middle-aged (45 to 55 years). Early detection, favorable localization in the brain and well-performed surgery bring good results to the patient in terms of improved outcome.

A retrospective study included patients with metastatic changes in the brain and meningiomas treated at the Clinic of Neurosurgery and the Clinic of Oncology of the University Clinical Center Niš in the period from the beginning of 2018 to the end of 2020.

By comparing the ratio of the presence of meningioma, as benign tumors in the brain, in relation to the percentage of secondary deposits present, we come to the conclusion that malignancy is on the rise. Early diagnosis and suspicion of this dissemination in primary metastasis enable surgical intervention followed by oncological treatment, which together prolong the patient's life.

Comparing the patients with meningioma who underwent surgery in the period from 2018 to 2020 according to gender, age structure, tumor localization, no statistically significant difference was observed. Females are ahead of males in terms of meningioma.

There has been an evident increase in malignancy in recent years in both genders. In meningioma, the treatment is surgical. In case of solitary changes after surgery, oncological treatment is performed. In the case of multiple changes in the brain, the treatment is oncological, which includes the use of radio and chemotherapy.

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Key words: *meningioma, multiple metastases, solitary metastasis, oncological treatment*

[1]University of Niš, Faculty of Medicine, Niš, Serbia
[2]University Clinical Center Niš, Clinic of Neurosurgery, Niš, Serbia

Contact: Vesna Nikolov
3/1 Prijezdina St., 18000 Niš, Serbia
E-mail: v.novak@yahoo.com

Introduction

The presence of malignant patients is evidently increasing in recent years. About 16% of the world's population is covered by registration systems that provide cancer incidence statistics, while mortality data are available for about 29%. The incidence and mortality from breast cancer vary significantly depending on the world region (1). The American Cancer Society, the Centers for Disease Control and Prevention, the National Cancer Institute, and the North American Association of Central Cancer Registry work together each year to update cancer rates and trends in the United States. This report updates statistics on lung, breast, prostate and colorectal cancer and highlights the use of selected surveillance data to help develop national cancer control plans (2).

The percentage of metastatic changes in the brain, as secondary deposits, is also increasing. Single or multiple changes in the brain are an increasingly common subject of treatment in neurosurgical institutions. The number of brain metastases is far higher than primary brain tumors. The most common sources of brain metastases are lung cancer, breast cancer and melanoma. While lung cancer and breast cancer are much more common, melanoma has the highest risk of spreading to the CNS among all common types of cancer. (3). Management of patients with brain metastases has become more important recently due to the increased incidence of these tumors and the prolonged survival time of the patient that accompanies the increased control of systemic carcinoma (4).

Fast lifestyle, irregular and improper diet, problems at work, numerous obligations, leave less and less time for an individual to take care of himself and dedicate time to his health. Antioxidants are important ingredients present in fruits and vegetables (FAV). With increased consumption of FAV in its raw and processed form, a predominantly plantbased diet rich in FAV could reduce the risk of developing malignant human diseases (5). There is ample evidence that FAV consumption is important for human health because it is rich in health-enhancing nutrients (6, 7).

All the unwanted symptoms are attributed to fatigue, a little time spent sleeping, and when things get more serious and when one dedicates time to oneself, one comes to the realization that it is a serious illness. Gavurova et al. state that environmental health is among the priority areas of public health and the current professional community is intensively dealing with it (8). Appeals for preventive examinations bear fruit and make one sets aside time for oneself and dedicates oneself to taking care of one's health. Asymptomatic metastases are increasingly being diagnosed through increased screening due to known risk factors, as well as mandatory imaging required during screening for many clinical trials of melanoma (9, 10). Symptoms caused by increased intracranial pressure, mass effect, impaired drainage of cerebrospinal fluid lead to focal deficit, weakness, numbness, imbalance, vision loss, behavioral changes, and often epileptic seizures (11). When the examinations start, it is understood that it is a serious health disorder. Sometimes frequent headaches, nervous insomnia lead the doctor to suspect that it is a change in the brain, so MSCT of the brain is indicated. In contrast to malignant diseases, the distribution of benign tumors, meningioma has been homeogenic for many years. Meningioma is characterized by slow growth, nonspecific symptoms related to the region in which they occur. Meningiomas are most often slow-growing, benign, primary intracranial tumors in adults, middle-aged, 45 to 55 years (12, 13). Early detection, favorable localization in the brain, and well-performed surgery bring good results per patient in terms of cure. Meningiomas are rare intracranial tumors in childhood and adolescence and account for 0.4-4.1% of all tumors in children (14). Data from the literature indicate that the highest incidence of meningioma is from the age of 45 to 55, and that it increases until the age of seventy (15). If the MSCT of the brain shows the presence of multiple mentions, the presence of secondary deposits is suspected. The incidence of multiple meningiomas ranges from 1–2% to 8% (16).

Brain MRI is a diagnostic procedure that determines the further course of treatment. Based on it, a further plan of neurosurgical treatment is performed, which is supplemented by the use of radiotherapy for multiple metastases.

Methods

A retrospective study was performed at the Clinic of Neurosurgery of the UCC Niš and the Clinic of Oncology of the UCC Niš in the period from the beginning of 2018 to the end of 2020. The following groups were monitored: a group of patients with malignant diseases and secondary metastases, and a group with a benign brain tumor, meningioma in the mentioned period.

Results

In the examined group, in the three-year period, there were 86 patients who underwent meningioma, among which there were 32 (37.2%) men and 54 (62.8%) women. There was no significant difference in age structure by sex ($p = 0.572$).

There was no significant difference in the distribution of patients who underwent meningioma surgery by sex according to the examined years ($p = 0.667$), or by age structure ($p = 0.448$). In relation to the distribution of meningioma localization, there was no statistically significant difference in the three examined years ($p = 0.882$). The number of deceased patients by age was uniform without significant difference ($p = 0.503$) (Table 1).

It was found that a significantly higher number of deceased patients had basal localization of meningioma ($p = 0.021$) (Table 2).

Table 1. Data by years of testing

		2018.	**2019.**	**2020.**	p
Gender	*m*	13 (43.3)	12 (35.3)	7 (31.8)	0.667
	f	17 (56.7)	22 (64.7)	15 (68.2)	
Age		66.07 ± 11.86	63.76 ± 8.27	67.00 ± 9.48	0.448
Region of brain	*Temporally*	10 (33.3)	6 (17.6)	5 (22.7)	
	Parietal	9 (30.0)	13 (38.2)	8 (36.4)	
	Basal	3 (10.0)	3 (8.8)	2 (9.1)	
	Frontally	8 (26.7)	12 (35.3)	7 (31.8)	0.882
Death		1 (3.3)	2 (5.9)	0 (0.0)	0.503

Table 2. Distribution of meningioma by localization and lethal outcome

Region of brain	Death		p*
	No	Yes	
Temporally	21 (25.3)	0 (0.0)	
Parietal	29 (34.9)	1 (33.3)	
Basal	6 (7.2)	2 (66.7)	
Frontally	27 (32.5)	0 (0.0)	0.021

* Fisher's test

In the three-year period (2018-2020), there were 319 patients in the examined population, including 111 (34.8%) patients with individual metastases, 122 (38.2%) patients with multiple metastases and 86 (27.0%) patients with meningiomas.

A significant difference in the distribution of tumor changes in the three-year period was found (χ^2 = 11,768; p = 0.019). Metastases were more prevalent in 2020, while in 2018 multiple metastases were the most prevalent. The distribution of meningioma is homogeneous over a three-year period (Table 3).

A significant difference in gender distribution was found among the examined groups (χ^2 = 13.487; p = 0.001). Single metastases were more common in men, while meningiomas were more common in women. Multiple metastases were equally present in both genders. There was no significant difference in age distribution (p = 0.513).

Mortality is highest in multiple metastases (χ^2 = 47,485; p < 0.001) (Table 4).

Table 3. Distribution of primary cancers and metastases by age

	2018.	**2019.**	**2020.**	p
Solitary metastases	35 (37.8)	40 (35.1)	36 (45.6)	
Multiple metastases	61 (48.4)	40 (35.1)	21 (26.6)	
Meningioma	30 (23.8)	34 (29.8)	22 (27.8)	0.019

Table 4. Gender and age distribution by groups

		Solitary metastases	Multiple metastases	Meningioma	p
Gender	*m*	70 (63.1)	68 (55.7)	32 (37.2)	0.001
	f	41 (36.9)	54 (44.3)	54 (62.8)	
Age		64.24 ± 10.41	63.63 ± 11.84	65.39 ± 9.93	0.513
Death		9 (8.1)	44 (36.1)	3 (3.5)	< 0.001

Discussion

The existence of malignancies in the world, as well as in our country, has been a current topic in recent years. Each year, the American Cancer Society estimates the number of new cancers and deaths expected in the United States in the current year and compiles the latest data on cancer incidence, mortality and survival based on incidence data from the National Cancer Institute, Centers for Control and Prevention (17). The fact is that the rate of benign changes in the brain, in stagnation, is homogeneous. By comparing the ratio of the presence of meningitis, as benign tumors in the brain, in relation to the percentage of secondary deposits present, we come to the conclusion that malignancy is on the rise.

Comparing the operated patients with meningioma, in the period from 2018 to 2020, according to gender, age structure, tumor localization, no statistically significant difference was observed in the examined three-year period. Following other literature, it has been noticed that females are ahead of males in terms of meningioma. Meningiomas are twice as common in women as in men (18). The annual incidence of meningioma is 2 to 7 per

100,000 women and 1 to 5 per 100,000 men (M: F = 1.8: 2.1) (19). Maja G. Et al. indicate that 85.4% of subjects had meningiomas localized in the anterior cranial fossa, while 14.6% of subjects had meningiomas in the posterior cranial fossa (20). A higher presence of meningiomas in the anterior cranial fossa was noted and in the study of Milenković and associates (21). According to data from the literature, most meningiomas are located on the convexity (35%), most often parasagittal, along the upper sagittal sinus (20%) (22). Regarding the localization of the meningioma in our series, the most common presentation of the tumor was in the parietal lobe (Table 2). The results of Bassiouni et al. show that meningiomas of the frontal (42%), temporal (54.5%) and parietal localization (70%) are more often found on the left side. The frequency of meningiomas on the right and left sides of the occipital region and cerebellum is equal (50%). Of the total number of subjects, 4.38% have meningiomas localized in sella turcica (23).

The number of deceased patients in regard to age was uniform (Table 1). Monitoring and comparing meningiomas according to localization showed that a significantly higher number of deceased patients had a basal presentation of the tumor (Table 2). This is explained by the fact that in this localization of the tumor, the operative approach itself is much more complicated, with a major disruption of important brain structures. Surgical treatment of skull-based meningiomas has changed radically in the last two decades. Extensive surgery for patients with basal meningioma is the main treatment; however, this is often challenging due to narrow surgical corridors and the proximity of critical neurovascular structures.

New surgical technologies, including three-dimensional (3D) preoperative imaging, neuromonitoring, and surgical instruments, have gradually facilitated the surgical resectability of these meningiomas, reducing postoperative morbidity (24). Also, basal meningiomas often develop local recurrences after surgical resection. Complete removal is difficult because these deep-rooted tumors involve critical neurovascular structures. Therefore, the treatment strategy for recurrent basal meningiomas remains controversial (25). With recent advances in surgical technology such as preoperative imaging, neuromonitoring, and surgical instruments, surgical resectability of intracranial meningiomas has increased in the last two decades (26).

Regarding secondary deposits, a significant difference was found by years in terms of the presence of one or more metastases. Namely, in 2020, there was a higher number of detected mono metastases than in 2018, when a higher number of multiple changes in the brain was recorded (Table 3). About 80% of brain metastases are supratentorial, while 15% are infratentorial or leptomeningeal, and 5% affect the brainstem itself. CT scans of the brain with and without contrast can detect most metastases ≥ 10 mm in the supratentorial region and most hemorrhagic lesions (27). However, magnetic resonance imaging (MRI) with and without gadolinium is far more sensitive, especially for smaller lesions, posterior fossa lesions, and lepto meningeal disease (28).

The lungs, breasts, and skin (melanoma) are the most common sources of brain metastases, and up to 15% of patients' primary sites remain unknown (29). Early diagnosis and suspicion of this dissemination in primary metastasis enable surgical intervention followed by oncological treatment, which together prolong the patient's life. The average survival time with brain metastases is usually less than a year, but when only isolated metastases (oligometastases) are found and can be treated, over 60 percent of people can survive two years or longer (30). Commonly prescribed treatments for brain metastases are surgery and/or radiation therapy. Optimal management of brain metastases remains controversial. Both whole-brain radiotherapy (VBRT) and local treatment or surgery (S) or radiosurgery (RS) are the cornerstones of treatment. Combination therapy can improve both overall survival and local control in patients with a single metastasis, but also leads to the benefit of local control in patients with two to four lesions (31).

Comparing the sex distribution, it was noticed that individual metastases are more present in males, while meningiomas are more common in females. Matthew et al. note that meningiomas are twice as common in women as in men (18). Kleihues et al. state that the annual incidence of meningioma is 2 to 7 per 100,000 women and 1 to 5 per 100,000 men. (19).

Multiple metastases are equally present in both sexes. There was no significant difference in age structure, while mortality was highest in multiple metastases (Table 4). Risk factors associated with melanoma metastases to the brain include male, mucosal or primary head and neck tumors, thick or ulcerated neoplasms, acral lentiginous or nodal lesions, and stage IV disease.

Current treatment strategies are unsatisfactory and brain metastases contribute to death in almost 95% of patients, with a median survival of less than 1 year despite treatment (32).

Certainly, improvements in imaging technologies during this period have led to increased detection of metastatic lesions; powerful neuroimaging modalities have become widely available and used to detect brain metastases, especially MRI, which is currently used to assess approximately 64% of cancer patients compared to 2% 20 years ago (33).

Conclusion

Females predominate in the percentage of meningioma in relation to males.

There has been an evident increase in malignancy in recent years in both genders.

MRI enables much better and more accurate diagnosis of the presence of secondary deposits in the brain.

In meningioma, the treatment is surgical.

In case of solitary changes after surgery, oncological treatment is performed.

In case of multiple changes in the brain, the treatment is oncological, which includes the use of radio and chemotherapy.

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TROGODIŠNJA UPOREDNA ANALIZA PRISUSTVA MENINGIOMA I SEKUNDARNIH DEPOZITA NA MOZGU

Vesna Nikolov[1,2], Boban Jelenković[1,2], Miša Radisavljević[1,2], Nikola Stojanović[2]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Univerzitetski klinički centar Niš, Klinika za neurohirurgiju, Niš, Srbija

Kontakt: Vesna Nikolov
Prijezdina 3/1, 18000 Niš, Srbija
E-mail: v.novak@yahoo.com

Oko 16% svetske populacije pokriveno je sistemima registracije, koji pružaju statistiku incidencije raka, dok su podaci o smrtnosti dostupni za oko 29% populacije. Povećava se i procenat metastatskih promena u mozgu, kao sekundarnih naslaga. Meningiomi su najčešće sporastući, benigni, primarni intrakranijalni tumori kod odraslih, srednjih godina, od 45 do 55 godina. Rano otkrivanje, povoljna lokalizacija mozga i dobro izvedena operacija donose dobre rezultate po bolesnika, u smislu izlečenja.

Retrospektivnom studijom, u periodu od početka 2018. do kraja 2020. godine, obuhvaćeni su bolesnici lečeni na Klinici za neurohirurgiju i Klinici za onkologiju Univerzitetskog kliničkog centra Niš, sa metastatskim promenama na mozgu i meningiomima.

Upoređivanjem odnosa prisustva meningitisa, kao benignih tumora u mozgu, u odnosu na procenat prisutnih sekundarnih naslaga, dolazi se do zaključka da je malignitet u porastu. Rana dijagnoza i sumnja na ovu diseminaciju u primarnim metastazama omogućavaju hiruršku intervenciju praćenu onkološkim lečenjem, što, zajedno, produžava život bolesnika.

Poređenjem operisanih bolesnika sa meningiomom, u periodu od 2018. do 2020. godine, prema polu, starosnoj strukturi i lokalizaciji tumora, nije uočena statistički značajna razlika u ispitivanom trogodišnjem periodu. Žene su ispred muškaraca, u pogledu meningioma.

Poslednjih godina, evidentan je porast maligniteta kod oba pola. Meningiom se leči hirurški. U slučaju solitarnih promena posle operacije, radi se onkološko lečenje. U slučaju višestrukih promena na mozgu, lečenje je onkološko, što podrazumeva primenu radioterapije i hemoterapije.

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Ključne reči: *meningiom, multiple metastaze, solitarne metastaze, onkološki tretman*

Original article

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CLINICAL-PATHOLOGICAL CHARACTERISTICS OF HORMONE INDEPENDENT LOBULAR BREAST CARCINOMA

Tamara Nikolić[1], Ivan Ilić[1,2], Maja Jovičić Milentijević[1,2], Nemanja Nikolić[3], Sara Stojanović[1], Marija Marinković[4], Milica Stanković[2], Aleksandar Milićević[2], Jelena Grujović[2], Nikola Petković[5], Velimir Perić[6], Jovana Nešić[7], Tomislav Kostić[1]

Estrogen has a role in the proliferation of luminal layer of epithelial breast cells and approximately 70% of human breast cancers have estrogen receptor expression. Based on the hormone receptor expression, we can classify these carcinomas as hormone-dependent and hormone-independent. Considering that the data in the world literature are incomplete, the aim of this research was a comparative analysis of these characteristics of hormone-dependent and hormone-independent lobular breast carcinomas. One hundred thirty-eight cases of lobular breast carcinomas were analyzed in relation to their hormonal status. Obtained morphometric values were subjected to statistical analysis using Student's t-test and Fisher's test. Statistically significant difference between groups of patients with hormone-dependent and hormone-independent lobular breast carcinomas was found for the age of patients ($p = 0.036$) and nuclear gradus ($p = 0.006$). On the other hand there was no statistically significant difference between two groups of patients considering the presence of metastasis in the axillary lymph nodes ($p > 0.05$). It was found that the patients with hormone-independent lobular breast carcinoma were significantly older then the patients with hormone-independent lobular breast carcinoma, and that expression of hormone receptors did not play a key role in metastasis of this carcinoma to the axillary lymph nodes.

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Key words: *hormone dependence, lobular breast carcinoma, lymph node status*

[1]University of Niš, Faculty of Medicine, Niš, Serbia
[2]University Clinical Center Niš, Center of Pathology and Pathological Anatomy, Niš, Serbia
[3]Polyclinic dr Nikolić, Niš, Serbia
[4]University Clinical Center Niš, Clinic of Pulmonary Diseases, Niš, Serbia
[5]University Clinical Center Niš, Clinic of Plastic and Reconstructive Surgery, Niš, Serbia
[6]University Clinical Center Niš, Clinic of Cardiosurgery, Niš, Serbia
[7]University Clinical Center Niš, Mental Health Center, Niš, Serbia

Contact: Tamara Nikolić
31/20 Cvijićeva St., 18000 Niš, Serbia
E-mail: tamarastojanovic1992@gmail.com

Introduction

Every year, about a million women in the world are diagnosed with breast cancer. Estrogen plays a role in the proliferation of the luminal layer of breast epithelial cells and the development of breast cancer. About 70% of human breast cancers show estrogen receptor expression (1, 2). Breast cancers have long been classified on the basis of numerous requirements as estrogen-dependent or estrogen-independent tumors (3). Estrogen is a transcription factor that regulates the genetic program of cell cycle progression and growth in the breast glands (2).

For more than three decades, ER has been the most important biomarker for breast cancer treatment, primarily because of the significant benefits of endocrine therapy for ER-positive but not for ER-negative tumors in women of all ages (4, 5).

The development of immunohistochemical methods using monoclonal antibodies has enabled the determination of hormone receptors, which is of particular importance for determining the biological potential of breast cancer. Assessment of hormone receptor expression in the nuclei of breast cancer tumor cells is a part of routine diagnosis and provides important information on prognosis and relevant therapeutic approach (6).

Hormone therapy reduces the relative risk of disease recurrence in more than 50% of patients with hormone-dependent breast cancer (7-9).

The aim

Since the data in the world literature are partial, a comparative analysis of the clinical and pathological characteristics of hormone-dependent and independent lobular breast cancers has been set as the goal of this research.

Materials and methods

Twenty-eight cases of lobular breast cancers diagnosed in the period from 2007-2009 were analyzed. The other 110 selected cases of lobular carcinomas that occurred in the same period were taken for the purpose of mutual comparison. Tissue samples of lobular breast cancers, obtained by excisional biopsies or mastectomy with axillary dissection, were used for this study. It is important to mention that all these patients were treated at the University Clinical Centre Niš. Samples were routinely processed in paraffin molds and archived together with pathohistological and clinical documentation at the Institute of Pathology of the University Clinical Center in Niš.

Paraffin molds were microtomically cut into sections of about 4 μm in thickness, adhered to "super frost" plates, and stained immunohistochemically for estrogen receptors (ER) and progesterone receptors (PR). After dewaxing and hydration of the samples through xylene and a series of decreasing concentrations of alcohol, the antigen was unmasked in a microwave oven, 20 min in citrate buffer, followed by cooling to room temperature, washing and blocking endogenous peroxidase with 3% hydrogen.

The samples were washed in PBS buffer (Phosphate Buffered Saline), pH = 7.4, and then the primary antibody was applied with an incubation of 40 min at room temperature. PBS lavage was followed by Labelled Streptavidin-Biotin 2 System, Horseradish Peroxidase (LSAB2 System-HRP, 15 ml, Code K0673), containing yellow and red LINK and incubated for 20 min, and PBS-lavage was performed between each step. Visualization was performed with DAB (Diaminobenzidine), followed by good rinsing with running water for 2 min, hematoxylin contrast, dehydration, and tissue incorporation with DPX. DAKO (Glostrup, Denmark) reagents were used.

The results of immunohistochemical analyzes of ER and PR expression were evaluated semi-quantitatively based on the Allred "scoring" system, where the total score was obtained by: sum of percentage involvement of tumor cell nuclei (score 1 - less than 1% of tumor cell nuclei; score 2 - of 1 up to 10% of tumor cell nuclei, score 3 - from 11 to 33% of tumor cell nuclei, score 4 - from 34 to 66% of tumor cell nuclei, score 5 - more than 67% of tumor cell nuclei) and score of nuclear staining intensity - weak, score 2 - medium, score 3 - strong). Tumors with a score of 2 or more were considered positive.

Patients with metastatic disease of the visceral organs, as well as patients with incomplete hormone-dependent lobular carcinomas (ER +/PR - and ER -/PR +) were not analyzed.

The obtained values for the examined parameters, such as age of patients, pT stage, histological grade, nuclear grade, axillary lymph node involvement in hormone-independent and hormone-dependent lobular breast cancers were subjected to the following methods: descriptive statistics (average value and standard deviation) and comparative tests (parametric (t-test) and non-parametric (Fisher's test)) type using GraphPad Prism version 5.03 (San Diego, CA, USA).

Results

Out of 138 examined patients with lobular breast cancer, 28 were not hormone dependent and 110 were hormone dependent. The average age of patients, as well as the average age of patients with ER and PR positive and negative scores are shown in Table 1. Based on the student's t-test, a statistically significant difference was found in the average age of patients with hormone-independent or hormone-dependent lobular cancer. The absence of immunohistochemical staining of tumor cell nuclei in hormone-independent lobular breast cancers is given in Figure 1. No statistically significant relation was observed for pT stage and histological grade of hormone-dependent and hormone-independent lobular breast cancers. Patients with hormone-independent lobular carcinoma of the breast (Figure 2) were statistically significantly more likely to have a high nuclear grade (NG III) compared to patients with hormone-dependent lobular carcinoma of the breast (60% vs. 28.18%), and significantly lower incidence of grade I and grade II (7.14% vs. 13.63% and 32.14% vs. 58.18%) (Fischer test, $p = 0.006$) (Table 1).

Statistical analysis of the relationship between the presence of metastases in axillary lymph nodes and hormonal status of lobular breast cancer showed no statistically significant difference (Fisher's test, $p = 0.187$), where patients with hormone-independent LCD were more likely to have metastases in axillary lymph nodes compared to patients with hormone-dependent LCD (25% vs. 21.8%) ($p > 0.05$) (Table 2).

Table 1. Average age of patients, pT stage, histological grade, nuclear grade of patients with hormone-independent and hormone-dependent lobular breast cancers

Parameter/Hormonal type LCD	**Hormone-independent**	**Hormone-dependent**	***p* value**
Number of cases	28	110	/
Average age	53.3 ± 9.9	51.1 ± 10.3	0.036
pT stage			
pT1	13	60	0.4561
pT2	10	35	
pT3	4	7	
pT4	1	8	
Histological grade			
I	2	9	0.1120
II	9	58	
III	17	43	
Nuclear grade			
I	2	15	0.006
II	9	64	
III	17	31	

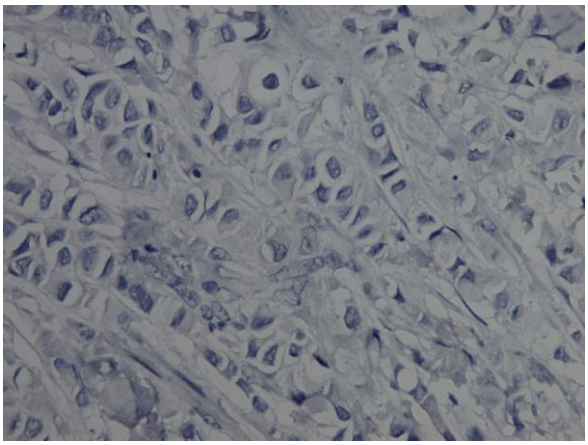

Figure 1. Negative estrogen receptor expression in the nuclei of hormone-independent lobular breast carcinoma (LSAB x 400)

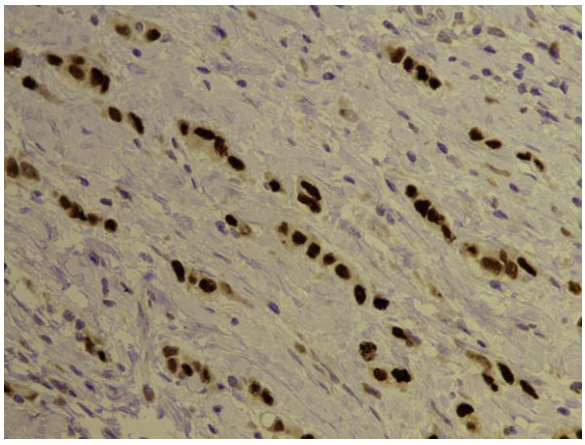

Figure 2. Positive progesterone receptor expression in hormone-independent lobular breast carcinoma (LSAB x 400)

Table 2. Relationship between hormone-dependent and hormone-independent lobular carcinomas and the presence of metastases in axillary lymph nodes.

Lymph node status	**Hormone-independent**	**Hormone-dependent**	Total	*p* value
Positive	7	24	31	0.187
Negative	21	86	107	
Total	28	110	138	

Discussion

Assessment of hormone receptor expression in the nuclei of breast cancer tumor cells is a part of routine diagnosis and provides important information on prognosis and relevant therapeutic approach (5, 6).

It is recommended that ER and PR receptor analyses should be considered positive if there is at least 1% of positive tumor cells on the tested samples in the presence of expected reactivity of internal (normal epithelial elements) and external control. The absence of benefits from endocrine therapy in women with ER-negative invasive breast cancer has been confirmed by detailed reviews of randomized clinical trials. Tumors showing less than 1% of positively stained tumor cell nuclei for ER or PR of any intensity should be considered negative, based on data that such patients do not benefit significantly from endocrine therapy (10).

Estrogen stimulates the growth and differentiation of ductal epithelium and the growth of intralobular connective tissue (11). Terminal ductulo/lobular breast units in young women are much more sensitive to estrogen, which has histamine-like effects on breast microcirculation, and the statistically significantly lower average age was in this study in patients with hormone-dependent lobular breast cancer ($p = 0.036$).

Although hormone receptor expression is associated with better ILC differentiation and lower nuclear grade, it can also be considered as an independent prognostic factor (12), which is consistent with our results that patients with hormone-independent lobular breast cancer statistically significantly more often have a high nuclear grade ($p = 0.006$). More importantly, however, ER and PR receptor expression is the most reliable predictive factor in response to endocrine therapy in breast cancer.

Some studies of patients with different subtypes of ILC do not show a significant difference in age, tumor size, growth pattern, lymph node status, and immunohistochemical expression of hormone receptors (13).

It is also considered that the prognostic value of standard pathological variables, such as tumor size, tumor grade and lymph node status, is significantly reduced in these patients with negative hormone receptors (14).

On the other hand, the importance of lymph node status in these patients cannot be determined with certainty, given that there are results that indicate the (non) existence of positive or negative correlation between negative expression of hormone receptors and lymph node status, Brouckaert et al., 2012). In this study, no statistically significant difference was found between the status of lymph nodes in patients with positive or negative expression of hormone receptors in lobular breast cancer ($p > 0.05$). Such results are possibly a consequence of the omission of patients with visceral metastases, as well as patients with incomplete hormone-dependent lobular carcinomas (ER +/PR - and ER -/PR +), which according to some authors may benefit from antihormone therapy (15).

Conclusion

By analyzing the clinical and pathological characteristics of hormone-dependent and hormone-independent lobular breast cancers in 138 patients, the following conclusions were drawn:

1. Patients with hormone-independent lobular breast carcinomas are significantly older than patients with hormone-dependent lobular breast carcinomas.
2. Patients with hormone-independent lobular breast carcinomas are statistically significantly more likely to have a high nuclear grade.
3. There is no significant difference between hormone-dependent and hormone-independent lobular breast carcinomas of patients in relation to the presence of metastases in axillary lymph nodes.
4. Understanding the relationship between hormone-dependent and hormone-independent lobular breast cancers in future studies could help find new targets in clinical "image" and pathohistological diagnosis, as well as drug development.

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KLINIČKO-PATOLOŠKE KARAKTERISTIKE HORMONSKI NEZAVISNIH LOBULARNIH KARCINOMA DOJKE

Tamara Nikolić[1], Ivan Ilić[1,2], Maja Jovičić Milentijević[1,2], Nemanja Nikolić[3], Sara Stojanović[1], Marija Marinković[4], Milica Stanković[2], Aleksandar Milićević[2], Jelena Grujović[2], Nikola Petković[5], Velimir Perić[6], Jovana Nešić[7], Tomislav Kostić[1]

[1]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[2]Univerzitetski klinički centar Niš, Centar za patologiju i patološku anatomiju, Niš, Srbija
[3]Poliklinika "Dr Nikolić", Niš, Srbija
[4]Univerzitetski klinički centar Niš, Klinika za plućne bolesti, Niš, Srbija
[5]Univerzitetski klinički centar Niš, Klinika za plastičnu i rekonstruktivnu hirurgiju, Niš, Srbija
[6]Univerzitetski klinički centar Niš, Klinika za kardiohirurgiju, Niš, Srbija
[7]Univerzitetski klinički centar Niš, Centar za zaštitu mentalnog zdravlja, Niš, Srbija

Kontakt: Tamara Nikolić
Cvijićeva 31/20, 18000 Niš, Srbija
E-mail: tamarastojanovic1992@gmail.com

Estrogen igra ulogu u proliferaciji luminalnog sloja epitelnih ćelija dojke i u razvoju karcinoma dojke. Oko 70% humanih karcinoma dojke pokazuje ekspresiju estrogenskih receptora. Na osnovu ekspresije hormonskih receptora, ovi karcinomi mogu se podeliti na hormonski zavisne i hormonski nezavisne. S obzirom na to da su podaci u svetskoj literaturi parcijalni, kao cilj ovog istraživanja postavljena je uporedna analiza ovih karakteristika hormonski zavisni i hormonski nezavisnih lobularnih karcinoma dojke. Analizirano je 138 slučajeva lobularnih karcinoma dojke u odnosu na njihov hormonski status. Dobijeni patohistološki, imunohistohemijski i klinički podaci upoređeni su studentovim t-testom i Fisherovim testom. Statistički značajne razlike između grupa bolesnica sa hormonski zavisnim i hormonski nezavisnim lobularnim karcinomom dojke pronađena je za starost bolesnica ($p = 0,036$) i nuklearni gradus ($p = 0,006$). Dok, sa druge strane, ne postoji statistička razlika između dve ispitivane grupe bolesnica u pogledu prisustva metastaza u aksilarnim limfnim čvorovima ($p > 0,05$). Nađeno je to da su bolesnice sa hormonski nezavisnim lobularnim karcinomima značajno starije od bolesnica sa hormonski zavisnim lobularnim karcinomom dojke, kao i da ekspresija hormonskih receptorane igra ključnu ulogu u metastaziranju ovog karcinoma u aksilarne limfne čvorove.

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Ključne reči: *hormonska zavisnost, lobularni karcinom dojke, status limfnih čvorova*

Original article

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PERCEPTIVE VOICE ANALYSIS IN CHILDREN WITH SPECIFIC LANGUAGE DISORDERS

Ivana Ilić Savić[1], Mirjana Petrović Lazić[1], Gordana Calić[2], Snežana Babac[1,3]

Specific language disorder (SLD) is a neurodevelopmental disorder that characterizes language deficits together with the preservation of some cognitive domains. Voice evaluation in children with SLD should indicate and encourage a new perspective and provide us with a conceptual approach that has the potential to better understand the voice system of children with SLD by completing a guide to voice rehabilitation in these children.

The aim of this research was to determine the components of the voice system, voice quality, as well as potential gender differences among children with SLD. The sample included 30 children with developmental dysphasia, aged 3 to 9 years (AS = 6.40; SD = 1.714), of whom 20 were boys and 10 were girls.

The adapted Quick Screen For Voice and GRBAS scale were used to assess the voice quality.

The obtained results show that the largest number of children with SLD have adequate biological predispositions for typical voice quality. A large number of children with SLD have no change in all analyzed perceptual voice parameters. The most common perceptual changes in the voice have been mild, followed by moderate, and the least common have been pronounced changes in the voice. There have been significant differences in the degree of hoarseness and hoarseness of the voice in relation to gender, more pronounced in boys ($p < 0.05$).

Although the changes in voice quality in children with SLD have been mild, they are potentially significant for vocal pathologists, pointing to the importance of prevention, which should be implemented at an early age in order to preserve a healthy and quality voice.

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Key words: *voice quality, specific language disorder, perceptual characteristics*

[1]University of Belgrade, Faculty of Special Education and Rehabilitation, Belgrade, Serbia
[2]Scholarship holder of the Ministry of Education, Science and Technological Development of the Republic of Serbia
[3]Clinic Hospital Center "Zvezdara", Belgrade, Serbia

Contact: Ivana Ilić Savić
2 Visokog Stevana St., 11000 Belgrade, Serbia
E-mail: ivana.ilic558@gmail.com

Introduction

Voice is a sound with which living beings are announced, and which is produced by special phonation organs (1). The voice is closely related to the sense of self-identity and is an indicator of health, emotions, age and gender of individuals (2). If they last longer, certain changes in the quality and duration of voice can potentially indicate the presence of a serious illness (3). Vocal cords are a source of voice that vibrates to periodically coagulate and dilute the air current (1).

Effective assessment of the voice in children requires knowledge and understanding of the anatomical and structural development of the phonation system during maturation. Scanning electron microscopy of children's larynx showed that there is a development of superficial and deep structures up to the age of 10, and a defined, layered structure of the characteristic lamina up to the age of 17 (4).

Previous research has shown that the most common voice disorders in children are dysphonia (impaired voice quality) and rhinophonia (impaired voice resonance). In most children, the voice naturally stabilizes after puberty, while in some it does not, according to some authors, as much as 15% does not show improvement in voice (5). So far, the findings so far have shown that children with dysphonia have a lower score on the scales of assessment of functionality in the social environment (6). Some authors point out that children with voice disorders respond to treatment, with voice weakness being the predominant disorder in children's voice pathology (7).

A study conducted in Brazil in which (N = 71) children aged 3 to 9 years participated showed that

most children had a slight deviation of the voice, with straining and instability when breathing as the main characteristics of the voice. Deviations of the fundamental frequency (F0) are correlated with stress and phonation. Shimmer and glottal cavities (GNE) are correlated with the general degree of voice deviation and the parameters of roughness, weakness and tension. In the mentioned research, it has been shown that the mean value (GNE) and (F0) in speech were the only measures that distinguished the voices in terms of the severity of the voice deviation. There was a connection between the perceptual and acoustic measures of children's voices. Children with a high voice had larger voice deviations (8).

Specific language disorder (SLD) is a neurodevelopmental disorder that characterizes language deficits with the preservation of some cognitive domains (9, 10). In these children, language difficulties include delayed onset and slower acquisition of lexical and grammatical forms, poorer vocabulary, and difficulties in acquiring and using flexible morphology and complex syntax. By definition, SLD is not a consequence of hearing loss, articulatory disorders, neurological diseases, or complex developmental disorders (11). Prevalence studies suggest that SLD has about 5-7% of school-age children (12). SLD thus differs from other disorders such as aphasia, intellectual disability, sensory impairment, and personality disorders (13). Children with SLD show poor oralpractical abilities, which as a result changes the nature of the vibration of the vocal folds compared to the vibration of the vocal folds in a healthy voice. Leonard (1998) points out that children with certain language deficits may misidentify certain speech elements due to their inherent "low phonetic substance", i.e. short-term low-intensity acoustics (14). Wright et al. point out that coarticulation or masking with longer, stronger vowels can contribute to poorer perception of previously produced consonant-vocal combinations (15).

Studies that have used auditory repetition and chronological judgment tasks show that children with SLD discriminate poorly or draw sequential conclusions about nonverbal stimuli, vowels, or consonants when stimuli are produced at a rapid pace or when they are short-term (16-18). In these studies, auditory repetition distinguished children with typical language abilities from children with SLD. During auditory repetition of stimuli, children with SLD exhibited abilities that were not directly related to verbal but to nonverbal abilities (19). Vowels caused the highest threshold of discrimination compared to other voice groups. Younger children (aged 3 to 5) with SLD showed elevated discrimination thresholds for vowels and complex tones compared to peers with typical language skills. Based on these results, the authors concluded that the spectral complexity rather than the phonemic nature of the stimulus affects auditory processing in children with SLD (20).

Analyzing the quality of voice in narrative speech, it has been noticed that in children with SLD there are pauses in narrative speech at the moment of difficulties in forming concepts, activating syntactic frameworks or processing syntactic and semantic information of lexical units (21, 22). Younger age groups of children with SLD show a tendency to produce speech pauses that differ in the number of vocal hesitations and in duration (measured by syllable) from the older age group (23).

Research comparing the quality of voice between the sexes in children with SLD shows that the difference between boys and girls in voice quality is significant. Girls have better results in prosody, which can be associated with better oralpractical abilities, while the boy's voice according to the authors sounds insufficiently mature for the age due to the lack of contrast of the tonal accent of the word (24). The results indicate that in most of these children, voice disorders do not disappear spontaneously with maturation (25).

Dobres et al. described the pathology of the larynx and its distribution in relation to age, sex and race in children. Data were collected based on the voice analysis of a large number of patients (N = 731) who reported to the children's hospital. It has been noticed that the most common pathologies of the larynx that affect the quality of the voice in children are subglottic stenosis, vocal nodules, laryngomalacia, functional dysphonia and vocal cord paralysis. In the overall sample, these pathologies were much more common in boys than in girls (26).

Checking the average adequate (expected) pitch, the authors found that most children with SLD have a changed pitch tone with voice interruptions in ascending/descending pitch, which can be explained by compensation for the ability to express appropriate melodic changes. along the sentence and inability to process intonation. To compensate for this deficiency, children increase the intensity of their voice in order to cause changes in the intonation of the sentence. Children with SLD tend not to use a pattern of declining intonation in their statements. Instead, they extend the final syllable to indicate the end of syntactic units. The appearance of reduced tilt in the voice in these children can be explained by the presence of motor impairment, since there is growing evidence that motor impairment is a common comorbidity in these children (27).

When assessing a child's voice, the importance of perceptual assessment of the voice is emphasized. Perceptual assessment is the most commonly used tool in the diagnosis and evaluation of the effectiveness of vocal treatment in everyday clinical environment, even in centers that have a rich selection of objective and subjective methods that can be used for the same purpose (5).

Although the principles of the therapy are largely similar to those distributed in adults, the strategy for voice rehabilitation in children should be different (28). The results of the research conducted by the Association of Vocal Pathologists in America indicate a significant connection between voice and psychosocial development of children, emphasizing the importance of voice testing at an early age in children of typical development and in children with speech and language disorders (29).

A large number of papers dealt with determining the language structure in children with SLD. The area of voice and voice disorders has been insufficiently studied in this population. Some authors have dealt with determining the acoustic characteristics of the voice and understanding prosody in children with SLD (16-24). Given the importance of perceptual assessment of voice, as pointed out by many authors in this field, this paper aims to determine the components of the voice system (respiration, phonation, resonance and range of voice) and voice quality in children with SLD. Also, the goal is to determine whether there are gender differences in the quality of voice in these children.

Method

Sample

The study included 30 children with SLD, aged 3 to 9 years (AS = 6.40; SD = 1.714). The sample consisted of 20 boys (66.7%) and 10 girls (33.3%). Respondents were uniform according to place of residence (city/village).

Procedure

The research was conducted at the Institute for Psychophysiological Disorders and Speech Pathology "Prof. Dr. Cvetko Brajović" in Belgrade. Before the beginning of the research, the approval of the Ethics Committee of the Institute for Psychophysiological Disorders and Speech Pathology "Prof. Dr. Cvetko Brajović" was obtained. After that, the parents of the children with SLD signed an informed consent for the respondents, and then the children themselves gave their consent. Only those children for whom the consent was obtained have been included. It has been explained to the children that they could give up the research at any time during its implementation. The examination was performed individually, in a quiet room intended for the work of a speech therapist in that institution. First, the children were given clear, concise and step-by-step instructions regarding the research procedure. All the children had the task to speak calmly, in a sitting position, spontaneously, recite a song, count. The period of conducting the research was from December 2018 to March 2019.

Tools

The adapted Voice Assessment Test (30) and the GRBAS Scale (31) were used in the study. At the very beginning of the Rapid Screening Test for Voice Assessment, there are general, socio-demographic data. A rapid screening test is used for subjective assessment of voice. The interrogation is conducted in an environment where there is silence. Respondents are asked to perform some of the verbal activities such as spontaneous conversation, imitation, verse recitation, counting. As a part of the screening test, the following four categories are assessed: respiration, phonation, resonance, and voice range. The GRBAS scale for subjective voice analysis assesses the voice of the examinees by three speech therapists who independently perform voice assessment. It is a standardized scale for subjective assessment of voice and the most common method of voice scaling that enables comparison and monitoring of voice. This scale contains five qualitative parameters of voice (G - overall impression of hoarseness, R - roughness in the voice, B - presence of breathiness in the voice, A - weakness in the voice, S - tension in the voice). Parameters were assessed on a four-point scale with a score of 0-3 (0 - normal voice, 1 - slight deviation, slight changes in voice, 2 - moderate deviation, moderately altered voice, and 3 - marked deviation, pronounced voice changes), while reciting a poem or reading a standard text.

Statistical data analysis

Descriptive and analytical statistics measures were used in statistical data processing. Frequency (number) and percentage (%) were used to assess the parameters related to the components of the voice system and voice quality, and the χ^2 test was used to examine the differences in voice quality in relation to gender. The results are shown in a table.

Statistical data processing was performed using the statistical processing package in the social sciences SPSS (SPSS, version 21.0).

Results

Respiration, phonation, resonance and voice range in children with SLD

The distribution of children with SLD according to the parameters of respiration, phonation and resonance are shown in Table 1.

The results show that 27 (90%) children with SLD had normal breathing necessary for speech, while in 3 (10%) children some difficulty in breathing were registered. These difficulties were wheezing when inhaling or exhaling (3.3%) and decreased volume or voice weakness (6.7%).

When it comes to the characteristics of phonation and the frequency of changes in vocal quality, the results of descriptive statistics show that four (13.3%) children with SLD had some difficulty in phonation, while 26 (86.7%) had normal speech. Difficulties were shown in the parameters of rough and tense phonation or hoarse voice (6.7%), vocal strain or effort (3.3%) and aphonia (3.3%).

From the aspect of resonance, 27 (90%) subjects had normal resonance, while 3 (10%) subjects had some deviation in resonance. These deviations were decreased nasality (3.3%) and increased nasality (6.7%).

The distribution of children with SLD according to the parameters related to the range of voice is shown in Table 2.

Table 1. Respiration, phonation and resonance parameters

	Respiration					**Phonation**									**Resonance**					
	Wheezing	Uneven breathings	Normal breathing	Limited breathing support	Decreased volume	Hoarse voice	Straining	Persistent lar.tones	Breathing quality	Aphonia	Coarse pharyngeal pressures	The highest point	Conversational voice	Too loud or soft	Normal quality	Decreased nasality	Mouth breathing	Increased nasality	Normal resonance	Nasal turbulence	Resonant characteristic
Number	1	0	27	0	2	2	1	0	0	1	0	0	0	0	26	1	0	2	27	0	0
%	3.3	0	90	0	6.7	6.7	3.3	0	0	3.3	0	0	0	0	86.7	3.3	0	6.7	90	0	0

Table 2. Parameters tone pitch, phonation period and high tone variation

	Tone pitch		**Maximum phonation time**			**Variation of high tone**		
	Normal	Modified	With in normal values	Below normal	Above normal	Small variations tone pitch	Voice interruption	Acceptable range
Number	29	1	19	11	0	0	1	29
%	96.7	3.3	63.3	36.7	0	0	3.3	96.7

The results show that tone pitch in 29 (96.7%) children was within normal values, while 1 (3.3%) respondent had a voice of altered pitch. When it comes to the maximum phonation time, in 19 subjects (63.3%) it was within normal values and in 11 subjects (36.7%) below normal values. When it comes to variations of high tones, there were almost none of them among children with SLD. The majority of the respondents 29 (96.7%) had an acceptable range of the highest tones and elasticity, while 1 (3.3%) respondent had a voice interruption at the ascending/descending pitch.

Perceptual characteristics of voice in children with SLD

The distribution of children with SLD according to the perceptual parameters of the GRBAS scale is shown in Table 3.

In relation to the level of hoarseness, 19 respondents (63.3%) had no change, while changes were registered in 11 (36.7%) children with SLD. Mild changes were the most common (20.0%), followed by moderate changes (10.3%), while pronounced changes in the voice were the least common (6.7%).

Voice roughness, as one of the perceptual parameters, was not registered in the voice of 21 (70%) children with SLD, while in 8 (30%) children it was. Mild changes were the most common (23.3%), while moderate and pronounced changes were significantly less common (3.3% each).

When it comes to breathiness in the voice, 19 (63.3%) children had no changes, while 11 (36.7%) had them. Mild changes were the most common (20.0%), followed by moderate changes (10.3%), while pronounced changes were the least common (6.7%).

The results of voice weakness examination showed that 14 (46.7%) children had no changes in voice weakness, while 16 (53.3%) children had them. Mild changes were the most common (30.0%), followed by moderate changes (16.7%), while pronounced changes were the least common (6.7%).

When it comes to voice tension, 16 (53.3%) children had no changes in voice tension, while 14 (46.7%) children had. Most often, these were mild changes (33.3%), followed by moderate and pronounced changes that were equally represented (6.7%).

Table 3. Perceptual parameters of the GRBAS scale

	Hoarseness				Roughness				Breathiness				Weakness				Voice tension			
	No change	Mild changes	Moderate	Expressed	No change	Mild changes	Moderate	Expressed	No change	Mild changes	Moderate	Expressed	No change	Mild changes	Moderate	Expressed	No change	Mild changes	Moderate	Expressed
Number	19	6	3	2	21	7	1	1	19	7	3	1	14	9	5	2	16	10	2	2
%	63.3	20	10	6.7	70	23.3	3.3	3.3	63.3	23.3	10.3	3.3	46.7	30	16.7	6.7	53.3	33.3	6.7	6.7

Perceptual characteristics of voice in relation to gender in children with SLD

Statistically significant differences in the perceptual characteristics of voice in children with SLD in relation to gender have been examined by the χ^2 test and are presented in Table 4.

Using the χ^2 test, statistically significant differences ($p < 0.05$) were obtained in two of the five examined perceptual characteristics of voice and sex: degree of hoarseness ($\chi^2 = 4.669$; df = 3; p = 0.039) and voice roughness ($\chi^2 = 4.571$; df = 3; p = 0.041). Mild changes in the degree of hoarseness of the voice were more frequent in boys (65%) than in girls (35%). Mild changes in voice roughness were more common in boys (70%) than in girls (30%). No statistically significant differences were found in breathiness of the voice and in sex ($\chi^2 = 2.729$; df = 3; p = 0.435); in weakness of the voice and in sex ($\chi^2 = 1.905$; df = 3; p = 0.206) and in tension of the voice and in sex ($\chi^2 = 2.831$; df = 3; p = 0.418). Changes in breathiness of the voice, voice weakness and voice tension were equally prevalent in boys and girls with SLD.

Table 4. Gender differences in perceptual characteristics of voice in children with SLD

Perceptual parameters	χ^2	df	p
Hoarseness	4.689	3	0.039*
Roughness	4.571	3	0.041*
Breathiness	2.729	3	0.435
Weakness	1.905	3	0.206
Voice tension	2.831	3	0.418

* significance at the level of $p < 0.05$

Discussion

The paper examines the quality of voice in children with SLD and potential gender differences between them.

By analyzing the obtained data in the field of respiration, we see that the largest number of children have normal breathing, while only a small percentage shows some difficulties, such as wheezing and weakness of the voice. The obtained results are in accordance with the results of some authors (8) who talk about difficulties in respiration in children of typical development, emphasizing that stress and instability during breathing are registered in most children.

The obtained results show that a small percentage of children with SLD have some difficulty in phonation, which manifests itself in the form of rough and tense phonation, vocal strain or aphonia, while most of the examined children have an adequate phonation, which is a prerequisite for normal speech. When it comes to the impact of resonance on voice quality in children with SLD, the research has shown that most children have normal resonance while a small percentage show a deviation in the form of decreased nasality or increased nasality. These findings are consistent with the previous research that points out that the most common voice disorders in children of typical development are dysphonia (impaired voice quality) and

rhinophonia (impaired voice resonance), indicating a correlation of fundamental frequency deviation (F0) with phonation and vocal straining (5, 8).

Analysis of the average, expected pitch with minimal variations in the range of voice, showed that most children with SLD had a normal pitch and acceptable range of highest tones and elasticity, while a small percentage of children had a pitch that was altered with voice interruptions at ascending/ descending pitch. The maximum phonation time in most children with SLD was within normal values. This result confirms the findings of the previous research which points out that most children with SLD have a tone of altered pitch with interruptions of voice at ascending/descending pitch which correlates with compensating for the ability to express appropriate melodic changes along a sentence and inability to process intonation. To compensate for this deficiency, children with SLD increase the intensity of the voice in order to cause changes in sentence intonation (27).

The obtained results show that there are no statistically significant differences in respiration, phonation, resonance and voice range in children with SLD in relation to gender. All examined parameters are equally represented in both sexes in children with SLD. The results of the previous research suggest that all pathologies of the larynx in children that affect voice quality (subglottic stenosis, vocal nodules, laryngomalacia, functional dysphonia and vocal cord paralysis) are much more common in boys than in girls (26).

By analyzing the obtained data, we have found that in children with SLD there are mild and moderate changes in voice quality (degree of hoarseness; hoarseness of voice; noise in the voice; weakness in the voice; tension in the voice). Mild changes are the most common, followed by moderate changes, while pronounced changes in the voice are the least common. The obtained results are in line with the previous research on voice quality, which confirms that most children have a slight deviation of the voice and that there are more frequent deviations in the voice in children with a high voice (8), and that voice weakness is the predominant disorder in pathology of the child's voice (7).

Analyzing the perceptual characteristics of the voice (degree of hoarseness; roughness of the voice; breathiness in the voice; weakness in the voice; tension in the voice) in relation to gender, statistically significant differences have been obtained for the two perceptual parameters. Mild changes in hoarseness and roughness of the voice are more frequent in boys, while changes in breathiness in the voice, weakness in the voice and tension in the voice are equally present in boys and girls with SLD. These results confirm previous research that points out that boys voices do not sound mature enough for the age, which is associated with a lack of contrast in the tonal accent of words, while girls have better results in prosody because they have better oral-practical abilities (24).

Restrictions

One of the limitations of this paper relates to the small sample. There is also a gender inequality of the sample, due to the generally higher tendency of boys in the population of children with SLD. The significance of the obtained results should further learn the results of future studies so that they can be generalized. Therefore, it is recommended that future research be directed towards larger samples, homogeneous in terms of gender and age. As we had even stronger strength, it would be good to compare children with SLD with children with other speech-language and voice disorders. Also, it would be desirable to explore additional instruments to process the voice system and voice quality.

Implications

Given the challenge that this research brings to speech therapy science and practice, the task for future research is to pay more attention to the quality of voice in the child population with speech and language disorders. Although difficulties have been reported in a small number of children, they have significant scientific and practical implications. They point to the importance of perceptual assessment of the voice performed by a vocal pathologist who has a trained sensibility for professional recognition of possible voice disorders. At the same time, they focus on the importance of preventive work with children with SLD and on vocal treatment, which is often neglected in the speech therapy practice with these children, yet it would be desirable to start with at an early stage in order to preserve a healthy and quality voice.

Conclusion

The research has been conducted with the aim of determining the components of the voice system, voice quality as well as potential gender differences among children with SLD.

Most children with SLD have adequate respiration and normal resonance, which are necessary for voice and speech. Most children have an acceptable range of the highest tones and elasticity, normal pitch tones and maximum phonation time within normal values. Difficulties in phonation, such as hoarse voice, vocal strain and aphonia, have been reported in a small number of children. All examined voice parameters (respiration, phonation, resonance and range of voice quality) have been equally represented in the examined boys and girls with SLD. Although in a smaller number, it has been noticed that in children with SLD there were mild and moderate changes in voice quality (degree of hoarseness; roughness of the voice; breathiness in the voice; weakness in the voice; tension in the voice). Changes in breathiness, voice weakness and voice tension were equally prevalent in boys and girls with SLD. Mild changes in the degree of hoarseness and roughness of the voice were more frequent in boys than in girls.

Voice evaluation in children with SLD should indicate and encourage a new perspective and to provide us with a conceptual approach that has the potential to better understand the voice system of children with SLD by completing a guide to voice rehabilitation in these children. Considering the importance of voice and speech for social development, the research findings imply the need for early treatment and support in order to preserve a healthy and quality voice in children with SLD.

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PERCEPTIVNA ANALIZA GLASA KOD DECE SA SPECIFIČNIM JEZIČKIM POREMEĆAJEM

Ivana Ilić Savić[1], Mirjana Petrović Lazić[1], Gordana Calić[2], Snežana Babac[1,3]

[1]Univerzitet u Beogradu, Fakultet za specijalnu edukaciju i rehabilitaciju, Beograd, Srbija
[2]Stipendista Ministarstve prosvete, nauke i tehnološkog razvoja Republike Srbije
[3]Klinički bolnički centar "Zvezdara", Beograd, Srbija

Kontakt: Ivana Ilić Savić
Velikog Stevana 2, 11000 Beograd, Srbija
E-mail: ivana.ilic558@gmail.com

Specifični jezički poremećaj (SJP) je neurorazvojni poremećaj, koji karakteriše jezičke deficite uz očuvanost nekih kognitivnih domena. Evaluacija glasa kod dece sa SJP treba da ukaže na novu perspektivu i podstakne put ka njoj, da nam pruži konceptualni pristup, koji ima potencijal za bolje razumevanje glasovnog sistema dece sa SJP, upotpunjujući vodič za rehabilitaciju glasa kod ove dece.

Cilj ovog istraživanja je da se utvrde komponente glasovnog sistema, kvalitet glasa, kao i potencijalne polne razlike među decom sa SJP. Uzorkom je obuhvaćeno 30 dece sa razvojnom disfazijom, uzrasta od 3 godine do 9 godina (AS = 6,40; SD = 1,714), od kojih 20 dečaka i 10 devojčica. Za procenu kvaliteta glasa korišćeni su adaptirani Brzi skrining test za procenu glasa (Quick Screen For Voice, Lee, Stemple & Galze, 2003) i GRBAS skala (Isshiki, Okamura, Tanabe & Morimoto, 1969). Dobijeni rezultati pokazuju da najveći broj dece sa SJP ima adekvatne biološke predispozicije za tipičan kvalitet glasa. Veliki broj dece sa SJP nema promene na svim analiziranim perceptivnim parametrima glasa. Najčešće perceptivne promene glasa su blage, potom slede umerene, a najmanje su zastupljene izražene promene glasa. Postoje značajne razlike u stepenu promuklosti i hrapavosti glasa u odnosu na pol; promuklost i hrapavost glasa izraženiji su kod dečaka ($p < 0,05$). Iako su promene u kvalitetu glasa kod dece sa SJP bile blage, one su potencijalno značajne za vokalne patologe, ukazujući na značaj prevencije, koju bi trebalo sprovoditi na što ranijem uzrastu, sa ciljem da se očuva zdrav i kvalitatan glas. Rad je nastao kao rezultat istraživanja u okviru projekta „Evaluacija tretmana stečenih poremećaja govora i jezika" (ON 179068) i „Socijalna participacija osoba sa intelektualnom ometenošću" (ON 179017), koji finansira Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije.

Rezultati našeg istraživanja pokazuju da najveći broj dece sa razvojnom disfazijom ima adekvatne biološke predispozicije za normalan govor podjednako zastupljene u oba pola. Najčešće perceptivne promene glasa su blage, potom slede umerene, a najmanje su zastupljene izražene promene glasa. Postoje značajne razlike u stepnu promuklosti i hrapavosti glasa u odnosu na pol, u korist dečaka.

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Ključne reči: *kvalitet glasa, specifični jezički poremećaj, peceptivne karakteristike*

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CONTINUING PROFESSIONAL DEVELOPMENT FOR PHARMACISTS: PREFERENCES, INTERESTS AND EXPERIENCE

Svetlana Stojkov[1], Jasmina Arsić[1], Branka Legetić[1], Nataša Jovanović Lješković[1]

One of the models of lifelong learning, Continuing Professional Development is a targeted, systematic, and tailored form of professional development. This paper aims to show the pharmacists' preferences, interests and experiences about models and areas of education.

Moreover, a cross-sectional observational study was conducted through an online survey posted on the website of the Serbian Chamber of Pharmacy. Besides, a questionnaire was created with 11 questions for the purpose of the research. The survey was completed by 565 pharmacists, and 93.4% were employed by a community pharmacy. More than half of the respondents (56.7%) felt they needed to develop their professional competences. In addition, for most pharmacists surveyed, direct interaction with the lecturer was significant. Over two-thirds of the respondents showed the highest affinity for the "case report", labelling it as "the most interesting", followed by workshops and lectures. The most interesting were the education via the Internet (57.7%), where the digital choices available on the Internet with the possibility of automatic podcast download were the first choice among half of the respondents. When asked about participation in continuing education (CE) the previous year, pharmacists responded that 95% had participated in CE, of which nearly half were at more than 5 CE.

According to the results of this research, the development of information technologies, the availability and diversity of educational content and models, the choice and active participation in education, were recognized by pharmacists in Serbia as an appropriate approach in professional development.

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Key words: *pharmacist, pharmacy education, continuing professional development, continuing professional development for pharmacists*

[1]University Business Academy in Novi Sad, Faculty of Pharmacy, Novi Sad, Serbia

Contact: Jasmina Arsić
5 Mladenaca Sq., 21000 Novi Sad, Serbia
E-mail: jasminabarsic@gmail.com

Introduction

Dynamic and constant changes in the areas of science, society, healthcare, and patient needs have created challenges for healthcare professionals, including pharmacists. One of the most significant challenges is adapting professional development to public needs, legislative requirements, and institutional systems. Educating healthcare personnel and the provision of continuing professional development (CPD) in the form of lifelong learning has become a focal point for interested healthcare personnel, healthcare systems, but also for the highest professional authorities and organizations, on a global scale (1, 2). Over the last decade, the pharmaceutical profession has changed aspects of the activity. From production, procurement, and issuance, distribution of medicines, counseling, disease prevention, and providing support to patients about self-medication as the dominant areas of competency. The primary role and, therefore, the training of pharmacists has shifted over the last 30 years in most European countries from the traditional "product focus" to the so-called "patient focus" approach. Economic factors, the availability of medicine to a broader population, uniformity, and quality preservation are some of the primary reasons as to why the production has shifted to large, most often semi-automatized systems operating within the pharmaceutical industry.

Additionally, pharmacists profiling and training has moved to the role of "medicinal use" professionals and therapy counseling. However, 21st century trends and rapid changes in all societal segments, including the pharmaceutical profession, have imposed and resulted in new as well as in bringing back into previous focus roles held by pharmacists. Personalized medicine, specific medici-

nal forms, the development of drugs used to treat rare diseases and deficiencies of certain medications for particular categories of patients will increase the need for small-scale production in compounding laboratories (3). This demand will require specific production know-how, skills, and quality control.

On the other hand, scientific developments in the fields of molecular genetics or biotechnology have led to significant discoveries such as genome mapping, gene editing, and a better understanding of the molecular mechanics of numerous diseases (4). Additionally, discoveries in fundamental science have enabled the development of new biological drugs, the development of gene therapy, and new drugs in cancer immune-therapy (CAR T-cell therapy) (5). We are living in the 4th industrial revolution, where the digital revolution has transformed and continues to change the way we live (6). Technologies developed in other areas have made their way into pharmacy and medicine. Smartphones, various apps, and wearables used to monitor health, virtual and augmented reality, information platforms, chatbots, virtual assistants, and many other examples of artificial intelligence applied in the healthcare system have had a significant impact on the future position and role of the pharmacist. New approaches to therapy, such as digital pills (7) or digital therapeutics (specially designed and tested therapy software) (8), have already gained FDA approval.

All this serves to expand the scope of information, skill, and know-how required by pharmacists so they can respond to the primary criterion of the profession - the pharmacist as an expert on medicinal drugs. Besides basic knowledge, there are three core domains in which the pharmacist must demonstrate expertise: knowledge of medication, human anatomy, and social behavior. It is making evident that a whole range of additional competences, skills, and "smart specialization" are needed. A commitment to lifelong learning is the foundation of successful professional development but also the survival of the pharmaceutical profession.

A new paradigm in the education of pharmacists – CPD

Extensive theoretical knowledge available to pharmacists, but with little opportunity to apply said experience in practice, attitudes that pharmacists are academic experts who lack self-confidence in decision-making when they have to use their expertise to the treatment of patients, and/or the opinion they experience difficulties in the practice of providing pharmaceutical healthcare (9), have all lead to a need for a stronger paradigm shift in terms of the pharmacist training. The traditional approach to educate these professionals is not an option. It has resulted in undesirable outcomes and the development of a new educational paradigm: training founded on competences and learning outcomes that reflect on the changes in behavior in the provision of medical/pharmaceutical care (10). The need for current educational programs, which include a behavioral psychology doctrine, has generated a need for new competencies (11), and the instruments for their development, such as a competency framework (12). Pharmacists are offered a new educational model that highlights an active approach, self-reflection, and the management of their professional development. Continuing Professional Development (CPD) is one such lifelong learning model. This model is an objective-based, systemic, and adaptable form of professional development where the participation of the healthcare professional may be regarded as a level of moral and professional accountability, and is expressed through expert assessment, ethics, attitudes, and values (13, 14). The CPD approach requires consideration of preferences and professional interests, activities chosen in response to identified needs, reflection and self-reflection, self-orientation, and the ability to adapt to change, i.e., the consistent improvement of competences (15).

Current features of continuing education for pharmacists in Serbia

Experiences gained in other countries in the assessment of the practices of pharmacists (16-18) have served to lay the groundwork in Serbia for a systemic and formal competency evaluation. A National Competency Framework was adopted in 2014 by an umbrella organization, the Pharmaceutical Chamber of Serbia (19), and is one of the first tools prepared to evaluate knowledge through applying specific indicators, i.e., competences. The research that followed indicates this instrument type offers the opportunity for assessment and the development and competency of pharmacists, as well as an incentive toward the professional development of pharmacists (20). The first assessment of pharmacists' competency in Serbia was conducted in 2011 aided by an adapted and validated Framework of overall levels (21), while the following assessment was completed throughout 2012/13 through a tailored and proved globally applied instrument (Global Competency Framework), where a discrepancy was noted between existing and required competencies, and a need to introduce more efficient learning models based on the practical application of knowledge and skills (22). The terms under which a license may be obtained and renewed in Serbia are defined by law (23-25) and for the most part, comply with other countries in the region. However, practice shows there is a need to adapt educational programs with pharmacists' requirements and preferences, and to provide support for CPD, i.e., to find solutions to potential barriers in the lifelong learning of pharmacists in practical terms.

This paper shall include pharmacists' preferences and attitudes of the members of the Pharmaceutical Chamber of Serbia, educational models, and factors which may have a positive or negative impact on education, i.e., professional development (field of education, models and instructional modalities, elements of time, Internet access). Based on their assessments, the surveyed pharmacists will

determine which competences they need to develop, areas they wish to know more about and to assess the importance of direct interaction with lecturers. The study includes information on the respondents' approach to continued education over the previous years.

Materials and methods

A cross-sectional observational study was conducted by researching online surveys available on the Pharmaceutical Chamber of Serbia's (FKS) website. For the needs of the research, learning models were created as were the factors which impact motivation for continuing education. The survey included questions relating to professional activities and the workplace, fields of professional interest, educational models and modalities, educational duration and days in the week best used for training, and the number of educational activities conducted over the previous year. By applying the Likert scale from 1 to 5 (1 - of least interest, 5 – of most interest), the surveyed pharmacists demonstrated their level of interest in various forms of training. The survey was anonymous and conducted voluntarily. The study included 565 participants, registered pharmacists - B.Sc. Pharm/M.Sc.Pharm.

Program package SPSS 20.0 was used for data entry and processing. For the needs of analysis and the description of the structure of samples per relevant variables, displays of frequency and percentages were used to show a representation of a specific category or response. Statistical characteristics of observation were processed through standard procedures and descriptive statistics for the comparative analysis of statistical features. Descriptive statistics data were represented in the form of arrhythmic mean, standard deviation, then frequency and percentage. Within the framework of comparative statistics, the single factor of variance with repeated measures (dependent measures) was used. In the applied tests, the threshold values of risk probability were significant, from 95% ($p < 0.05$) (statistically significant difference in parameters) and 99% ($p < 0.01$) (statistically highly significant difference in settings).

Results

An analysis of the data showed that over 90% of the respondents (pharmacists) engage in professional activities in the field of healthcare. Most respondents are pharmacists employed in the public sector (state-owned pharmacies), while pharmacists working in hospitals made up 4.4% of those surveyed. Such a structure corresponds with the formation of Pharmaceutical Chamber of Serbia members: according to data available from this organization, over 90% of its members were licensed pharmacists working in primary state-owned healthcare institutions/pharmacies at the initial start of the research, and of these, 4.1% were employed in the hospital sector (26). Regarding three areas of competency, defined in the National Competency Framework, pharmacists working in primary healthcare in Serbia (19), the surveyed pharmacists predominantly choose the development of expertise (competency) (Table 1).

Table 1. Professional activity and current positions vs. competence that requires development

	Number (%)
Categories that define your professional activity	
Public Health	525 (94.8%)
Production	5 (0.9%)
Research	3 (0.5%)
Academy	4 (0.7%)
Policy	10 (1.8%)
Other	7 (1.3%)
Describe your current position	
Public pharmacy - public or private	511 (93.4%)
Hospital pharmacy	24 (4.4%)
Industry	1 (0.2%)
Other	11 (2.0%)
What area of competence do you think you need to develop?	
Professional competences	303 (56.7%)
Organization and management competencies	90 (16.9%)
Professional and personal competences	141 (26.4%)

Besides expertise (competency), those factors which may impact choice and attractiveness regarding training, we also include modalities tied to education that provides for the presence of a lecturer (Face-to-Face Learning), with more or less interaction. Research results show that for most respondents, direct communication with a lecturer is essential, while less than one-fifth of the respondents view such interaction as insignificant. In the section of the survey where preferences are researched (Table 2), according to specific learning models on a scale of 1 to 5, over two-thirds of the respondents demonstrated the highest affinity towards the "case report" highlighting it as being "most interesting" followed by workshops and lectures. The arrhythmic mean and standard deviation are given in Table 2.

An overview of Table 3 shows there is a statistically significant difference in the average score on various learning styles - Wilks's lambda = 0.531, F (4, 490) = 108.07, p < 0.001, partial Eta squared = 0.469.

Through single factor analysis of variants of repeated measures (Table 3), the provided alternative responses to the question "What learning method suits you best?" were compared.

Table 2. Which learning method is best for you?
(frequencies and percentages) (1-least interesting; 5-most interesting); Arithmetic means and standard deviations

Learning method	Which learning method is best for you? Number (%)					Score/test	
	1	2	3	4	5	Arithmetic mean	Standard deviation
1. Lectures	47 (9.0%)	49 (9.4%)	117 (22.3%)	153 (29.2%)	158 (30.2%)	3.64	1.21
2. Case Study - Discussion	17 (3.2%)	27 (5.1%)	35 (6.6%)	119 (22.5%)	330 (62.5%)	4.41	0.95
3. Workshops	24 (4.6%)	47 (9.1%)	86 (16.6%)	143 (27.7%)	217 (42.0%)	3.95	1.14
4. Panel discussions	33 (6.5%)	84 (16.5%)	154 (30.3%)	155 (30.5%)	82 (16.1%)	3.35	1.12
5. Review	34 (6.7%)	52 (10.3%)	118 (23.3%)	190 (37.5%)	113 (22.3%)	3.58	1.14

Table 3. Significance of the model

	Value	F	Hipothetical df	Error df	p-level	Partial Eta square
Wilks' Lambda	0.531	108.07	4	490	< 0.001	0.469

Subsequent comparison conducted helped by a post-hock test (Table 4) exhibits that the individual learning style, i.e., the scores for each, less the score for alternative forms of Demonstation and Lecture, statistically significantly different. It is evident that the average achievement for the modality "Case Report" – Discussion is highest, followed by Workshops as the preferred modality, while Panel Discussions had the lowest average score. In other words, while the "Case Report" – Discussion method of learning is most preferred, the Panel Discussion method was shown to be the least favorite.

In choosing an educational modality, the most interesting were identified as online forms of training (57.7%), where the first choice of half of the respondents were Digital formats available on the Internet with the option to download podcasts. Internet access was found to be a significant limiting factor for 12.1% of the respondents. Of the other forms of "distance learning", we will mention CD/DVD, where approximately one-fifth of the respondents claimed this form of learning as their first choice, similar to live-stream webcasts and on-demand webcasts, which were indicated by almost 17% of pharmacists as their first choice.

A nonparametric chi-square test was performed to examine whether there is a statistically significant difference between the respondents who increasingly prefer interaction with a lecturer compared to those who deemed this form of communication as insignificant (less preferred), regarding the learning modalities they most prefer (Table 5).

A statistically significant difference was identified between respondent groups for the following learning modalities: Internet/online (χ^2 = 8.67; p = 0.013); CD/DVD (χ^2 = 6.83; p = 0.033); Interactive Workshops (χ^2 = 12.32; p = 0.002); Digital formats available on the Internet with the option to download podcasts (χ^2 = 7.39; p = 0.025). As shown in Table 5, the percentage of respondents who consider direct interaction with a lecturer as unimportant is somewhat higher than the percentage of those who claimed the Internet/online modality as their first choice (70%) compared to the group of respondents who consider that direct interaction with a lecturer is very important to their learning process, and to whom the Internet/online modality is the first choice is the case approximately 55% of the time. In terms of the modality that includes the use of CD/DVDs, the number of respondents who consider direct interaction with a lecturer as unimportant is slightly higher than those who claimed this modality of learning as their first choice (approx. 36%). This is opposed to the group of respondents who consider interaction with a lecturer as very significant and where this modality was chosen as the first choice in approximately 20% of cases. Furthermore, digital formats available on the Internet with the possibility of downloading pod-casts as the first choice is mostly preferred by those respondents who consider interaction with a lecturer to be insignificant to their learning process (approx. 63%). In the second group, this ratio is somewhat lower and amounts to 36%. On the other hand, a significantly higher number of respondents who prefer interaction with a lecturer also prefer interactive workshops as a learning modality, i.e. claimed workshops as their first choice (approx. 48%), which is opposed to the other group who chose this modality as first choice in approximately 26% of cases.

Time factor analysis included questions about scheduling, i.e., days in the week perceived to be most suitable for participating in educational programs. Half of the pharmacists chose Saturday as their first choice (49.4%), while Sunday was deemed third (last) option by 40.8%. Regarding duration, over half of the respondents chose one hour as their first choice, while somewhat less than half of the respondents chose half-day training. The question on CPD participation over the last years, the pharmacists responded that 95% had participated in CPD, of these almost half claimed to have attended over 5 CPD sessions, while only 5% of those surveyed claimed to have not attended a single CPD session (Figure 1).

Table 4. Post hock test with Bonferroni correction

		The difference AS (I-J)	Standard error	p-level
1	2	-0.767*	0.064	0.000
	3	-0.308*	0.073	0.000
	4	0.291*	0.070	0.000
	5	0.065	0.071	1.00
2	1	0.767*	0.064	0.000
	3	0.460*	0.049	0.000
	4	1.059*	0.053	0.000
	5	0.832*	0.060	0.000
3	1	0.308*	0.073	0.000
	2	-0.460*	0.049	0.000
	4	0.599*	0.052	0.000
	5	0.372*	0.064	0.000
4	1	-0.291*	0.070	0.000
	2	-1.059*	0.053	0.000
	3	-0.599*	0.052	0.000
	5	-0.227*	0.047	0.000
5	1	-0.065	0.071	1.00
	2	-0.832*	0.060	0.000
	3	-0.372*	0.064	0.000
	4	0.227*	0.047	0.000

Table 5. Favorite learning modality versus preference for direct interaction with the lecturer (interaction with the lecturer is not significant and significant)

Which learning modality is best for you?	How important is direct interaction with the lecturer to your learning?		χ^2	p
	Not significant number (%)	Significant number (%)		
1. Conference			2.14	0.342
I choice	22 (35.5%)	146 (42.2%)		
II choice	18 (29.0%)	108 (31.2%)		
III choice	22 (35.5%)	92 (26.6%)		
2. Internet/"online" CE			8.67	0.013
I choice	62 (70.5%)	202 (55.2%)		
II choice	19 (21.6%)	93 (25.4%)		
III choice	7 (8.0%)	71 (19.4%)		
3. Teleconference			0.242	0.886
I choice	2 (5.3%)	15 (7.4%)		
II choice	13 (34.2%)	71 (34.8%)		
III choice	23 (60.5%)	118 (57.8%)		
4. CD/DVD			6.83	0.033
I choice	18 (36.0%)	50 (19.8%)		
II choice	15 (30.0%)	110 (43.5%)		
III choice	17 (34.0%)	93 (36.8%)		
5. Interactive workshops			12.32	0.002
I choice	16 (26.2%)	167 (48.4%)		
II choice	25 (41.0%)	117 (33.9%)		
III choice	20 (32.8%)	61 (17.7%)		
6. Digital formats available on the Internet with the ability to download podcasts automatically			7.39	0.025
I choice	47 (63.5%)	154 (46.5%)		
II choice	18 (24.3%)	105 (31.7%)		
III choice	9 (12.2%)	72 (21.8%)		
7. Live stream webcasts			2.13	0.345
I choice	10 (25.0%)	31 (15.7%)		
II choice	18 (45.0%)	95 (48.0%)		
III choice	12 (30.0%)	72 (36.4%)		
8. On-demand webcasts			1.29	0.525
I choice	8 (22.2%)	31 (16.3%)		
II choice	18 (50.0%)	90 (47.4%)		
III choice	10 (27.8%)	69 (36.3%)		

χ^2 - a chi-square; p - the p-value or probability value

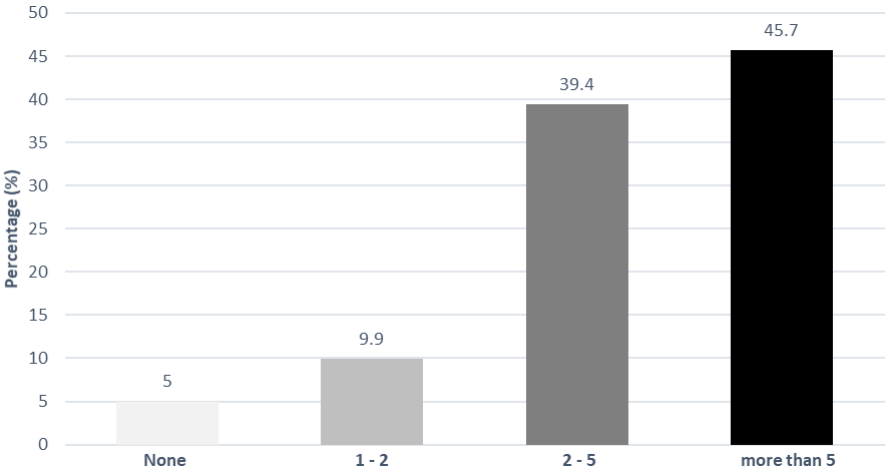

Figure 1. How many continuing educations did you participate in last year? Response distribution (frequencies and percentages)

Discussion

Identifying the need for training through the self-reflection and self-assessment of competences presents an excellent challenge for pharmacist practitioners (27, 28), as a kind of active attitude toward one's own professional development in which pharmacists can directly participate in creating and implementing a learning plan, i.e., managing their professional development which in turn will allow for better results in terms of professional growth. When participants identify gaps in their practical work or education, they can then target their objectives and create plans to improve the practice of pharmaceutical medicine with measurable results (29, 30). In our research, over half of the respondents considered it necessary to develop their expertise (competency), which may implicate that they have identified their weaknesses concerning skill. Research conducted earlier among pharmacists working in publicly-owned pharmacies in Serbia, in the study of evaluation and self-evaluation, showed that among three competency clusters, the least developed were precisely expertise or competency (20), which supports the assumption that pharmacists have developed the capacity to assess training needs in certain areas. The results of our study are very similar to the results obtained by the survey conducted by Driesen et al. (31), where the highest number of respondents consider the development of expertise (competency) as the most significant motivational factor in terms of CPD. When asked about the significance of developing the overall skill of an organization and management, our respondents' answers were similar to those of the mentioned study, i.e., they considered development in this area to be of a lesser priority.

Furthermore, concerning the indicated affinity toward active participation and discussion with a lecturer giving priority to CPD programs involving a dynamic approach to training, the results are consistent with our research. On the other hand, the respondents in our study considered workshops to be of most considerable interest, followed by case reports, while lectures were ranked; third, in contrast to Driesen et al., where lectures were ranked first place (31). The study conducted by Namara et al. 2009 (32), also showed that Australian pharmacists prefer face-to-face interaction with a lecturer as the most interesting CPD model.

Research shows that education involving more interactivity between lecturers and the audience, where there is a dialogue, which allows the audience to ask questions and confront opinions, enables critical thinking and developing problem-solving and decision-making skills (33). Traditional education, through lectures "ex-cathedra", does not provide for the expected effect, i.e., it has been shown to improve participants' knowledge, hence a subjective feeling of satisfaction, however, has a minimal impact on clinical practice and patient outcomes (34). Learning interactive activities, i.e., apply multiple learning methods (case-based learning, demonstrations, feedback, simulations, or patient role-playing), lead to more positive outcomes (35). Simulation is likely to play a more significant role in CPD because it allows for pharmacists to practice their skills and skill development in a safe environment, which supports learning based on competency (36, 37). Developments in the IT sector have enabled the modernization and greater availability of a variety of educational programs, which is reflected in the pharmacists who participated in our study in that a small percentage (approx. 12%) view Internet access as a limiting factor.

Similarly, other studies (31, 32) show that thanks to the Internet, CPD programs are now accessible also to pharmacists working in rural areas. Today, the educational applications on offer include training materials, including web practice and video recordings of interviews with patients, yet studies which evaluate the effects of different models of education continue to demonstrate that "live" training still has priority among pharmacists, in particular, the "case report", which was also confirmed by the results of our research. Other factors with an impact on the effect of learning include participant numbers, duration, and the complexity of the expected behavior of pharmacists while providing healthcare (30). It is imperative to establish a balance between pharmacists' preferences and training outcomes. Although there are several models for evaluation, a generally accepted set of standards has yet to be found that will allow one to measure the effectiveness of various educational models (38).

The amount of time that pharmacists from our study are willing to set aside for one training session, and the data stating that for 51.8% of those surveyed, time is the most limiting factor, implicates a potential barrier to CPD, considering that legislation links the amount of time spend on education and the number of required points to obtain a license. According to a study conducted in Scotland (39), as many as 9.8% of participants said that they do not have time for CPD, similar to Australian pharmacists (40), where, in addition to a lack of time, other barriers to CPD were identified as follows: a lack of motivation, availability (distance, cost), relevance and quality of educational content. Nonetheless, data obtained from our study, i.e., almost half of the surveyed pharmacists who participated in over 5 CPD sessions in the previous year, speaks of the fact that the time limit is being overcome. Research conducted in other countries indicates that the average amount of time spent on educational gatherings yearly is between 1 and 3 weeks (41), whereas the regulatory requirements referring to the number of training hours differ. In the United States, these are set at approximately 15 hours per year (2). According to research conducted by the International Pharmaceutical Federation (FIP), the average number of hours pharmacists spend per year on training is approximately (40), while the same study indicates that 9.8% of the respondents did not participate in CPD programs (42). In comparing these results with the results of our research, we can see that the number of pharmacists from our study who did not attend any training over the previous year is almost less than half that amount.

Conclusion

One crucial precondition of quality healthcare and patient safety is the competency of healthcare personnel. For this to be ensured, it is necessary to provide expert and technical prerequisites, and learning models that comply with the needs and possibilities of practitioners. The aim of this article is not to pessimistically summarize the future survival of the pharmaceutical profession but rather to show that for pharmacists, there is a bright and exciting future but one that will require pharmacists to be ready for. During their academic studies, pharmacists receive sound knowledge on both medication and the human body, but they also receive sound so-called STEM education: Science, Technology, Engineering, and Mathematics. According to many analysts, these disciplines are fundamental to the skills needed for the workforce of the future (6).

According to the results of this research, the development of information technology, availability, and variety of educational programs and models, i.e., the possibility of choosing and actively participating in education have been recognized by Serbia's pharmacists as a desirable way to go when it comes to professional development. Quality, multi-disciplinary knowledge, and a willingness to engage in lifelong learning are the keys to the development of the pharmaceutical profession. Of course, accountability and obligation do not only fall on the pharmacists themselves but also educators, i.e., the educational system and on society as well.

The academic community must adapt their programs to the development of the field and society, to introduce new learning technologies and methodologies. Problem Based Learning and acquiring functional know-how should be the foundation from which the development of future curriculums is built. Moreover, to accept that it is impossible to establish a "one model fits all" curriculum in advance, but to have an understanding that educational programs should offer enough in the way of general knowledge but also prepare students for lifelong learning and introduce them to new skills such as digital literacy or innovative entrepreneurship.

On the other hand, each country should consider the regulation of the healthcare system and support the role of the pharmacist as an essential partner in developing a healthy society. Recognizing pharmacists' preferences in educational content and models based on self-evaluation and individual choice will have a positive impact on motivation and are an excellent basis to start from in terms of adapting models and tools used in continued education, which lead to more efficient training. Learning that is geared toward better clinical outcomes is based on a model, content, and duration in line with modern information, while at the same time being sufficiently attractive in motivating pharmacists to change, i.e. to improving both service provision and their education, and not the formal fulfillment of legal requirements for licensing.

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KONTINUIRANI PROFESIONALNI RAZVOJ FARMACEUTA U SRBIJI: PREFERENCIJE, INTERESOVANJA I ISKUSTVA

Svetlana Stojkov[1], Jasmina Arsić[1], Branka Legetić[1], Nataša Jovanović Lješković[1]

[1]Univerzitet Privredna akademija u Novom Sadu, Farmaceutski fakultet, Novi Sad, Srbija

Kontakt: Jasmina Arsić
Trg mladenaca 5, 21000 Novi Sad, Srbija
E-mail: jasminabarsic@gmail.com

Kao jedan od modela učenja kroz ceo život, kontinuirani profesionalni razvoj predstavlja ciljani, sistemski i prilagođeni oblik profesionalnog razvoja. Cilj rada je prikaz preferencija modela i oblasti edukacije, kao i interesovanja i iskustava farmaceuta u okviru istih.

Opservaciona studija preseka sprovedena je putem onlajn ankete objavljene na veb stranici Apotekarske komore Srbije. Pored toga, napravljen je upitnik, koji se sastojao od 11 pitanja, u svrhu istraživanja. Anketu je popunilo 565 farmaceuta, od toga 93,4% zaposleno je u javnoj apoteci. Više od polovine ispitanika (56,7%) smatra da je potrebno da razviju stručne kompetencije. Pored toga, za većinu anketiranih farmaceuta značajna je direktna interakcija sa predavačem. Preko dve trećine ispitanika pokazalo je najveći afinitet prema „prikazu slučaja", označivši ga kao „najinteresantniji" metod, zatim slede radionice i predavanja, kao metode edukacije.

Kao najinteresantnije pokazale su se edukacije putem interneta (57,7%), kod kojih su kao prvi izbor kod polovine ispitanika odabrani digitalni formati dostupni na internetu, sa mogućnošću automatskog preuzimanja podkasta. Na pitanje o učešću u kontinuirarim edukacijama (KE) prethodne godine, farmaceuti su odgovorili da je 95% učestvovalo u KE, od toga skoro polovina na više od 5 KE. Prema rezultatima ovog istraživanja, razvoj informacionih tehnologija, dostupnost i raznovrsnost obrazovnih sadržaja i modela, izbor i aktivno učešće u obrazovanju, farmaceuti u Srbiji prepoznali su kao odgovarajući pristup u profesionalnom razvoju.

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Ključne reči: *farmaceut, obrazovanje farmaceuta, kontinuirani profesionalni razvoj, kontinuirano profesionalno usavršavanje farmaceuta*

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PROGNOSTIC SIGNIFICANCE OF SERUM ALBUMIN IN DIFFUSE LARGE B-CELL LYMPHOMA IN THE PRERITUXIMAB AND RITUXIMAB ERA

Ljiljana Tadić[1], Nikola Krstić[2]

Diffuse large B cell lymphoma (DLBCL) is the most frequent subgroup of non-Hodgkin lymphoma. The aim of the author was to verify the existence of important pretreatment serum albumin (SA) level as an independent factor of disease prognosis in patients with DLBCL in the territory of Southeast Serbia, in the era of prerituximab and rituximab.

A total of 55 patients with DLBCL (R-CHOP group) and 14 patients (CHOP group) were included in the study. Patients were divided into 2 groups according to the value of pretreatment SA: SA 30 g/l and ≤ 30 g/l. We analyzed the correlation of SA value with clinical stage and age, as well as the survival of patients with DLBCL compared to SA according to a therapeutic protocol.

There was no significant correlation of SA with age ($p = 0.630$), clinical stage ($p = 0.943$) and survival ($p = 0.638$) in CHOP group. There was significant correlation between SA levels with survival ($p = 0.001$) in R-CHOP group. No significant correlation of SA with age ($p = 0.141$) and clinical stage of disease ($p = 0.305$). There was no significant difference in survival compared to the value of SA in CHOP group/Log-rank = 0.782. There was significant difference in survival compared to the value of SA in R-CHOP group/Log-rank = 0.002.

The relationship between the predictive value of SA and the treatment protocol for DLBCL evaluated by the logistic regression analysis showed that the level of SA was not a significant predictor for the choice of treatment (Wald = 1.540, $p > 0.05$)

Our research has confirmed a negative predictive value of pretreatment serum albumin levels in patients with DLBCL treated according to R-CHOP protocol. Retrospective studies with a larger number of DLBCL patients who were treated with CHOP protocol, would give more significant results for the predictive importance of SA. Prognostic indexes, which as part of the point system include the value of SA, can be very useful in predicting patients with DLBCL.

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Key words: *DLBCL, albumin, R-CHOP, CHOP*

[1]Military Hospital Niš, Department of Internal Medicine, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Niš, Serbia

Contact: Ljiljana Tadić
66 Dr Zorana Djindjića Blvd., 18000 Niš, Serbia
E-mail: mitanik19@gmail.com

Introduction

Diffuse large B cell lymphoma represent a diffuse proliferation of large neoplastic B-lymphocytes and is the most frequent subgroup of non-Hodgkin lymphoma.

It is a heterogeneous group of diseases with a spectrum of clinical, biochemical and immunohistochemical markers that have their own prognostic significance. Immunochemotherapy changes the approach in the prediction of patients with DLBCL. The value of albumins in the serum (SA) marker with potential prognostic significance in patients with DLBCL and may be considered as a surrogate for catabolic processes that occur within tumor affected organism. The inflammatory response in the malignant disease, is associated with a reduced concentration of SA and loss of cell mass. It is hypothesized that the increased demand for specific amino acids is necessary for the production of mediators of the immune response that are mobilized from available proteins, including albumins (1). Loss of body weight is traditionally associated with poor prognosis of disease (2).

The aim of the study was to identify the impact of pretreatment SA level on prognosis in patients with DLBCL in a real situation in the territory of southeast Serbia and compare its predictive value between patients treated in the era of prerituximab and those treated with rituximab containing therapy.

Methods

Demographics of 55 patients with DLBCL, including age, Ann Arbor clinical stage, and SA were collected. They were all treated by therapeutic protocol R CHOP (rituximab, cyclophosphamide, doxorubicin chydrochloride, vincristine, prednisolone), according to the standard procedure in the period 2009 to 2013 at the Clinic of Hematology and Clinical Immunology, University Clinical Center in Niš, Serbia. Patients included in this study were newly diagnosed DLBCL, previously untreated , age 24-82 years. An overview was made by 31 December 2015.

The historical control group included 14 patients with DLBCL age 41-75, treated during the period from 1991 to 2002 according to the protocol CHOP (cyclophosphamide, doxorubicin chydrochloride, vincristine, prednisolone), at the Clinic of Hematology and Clinical Immunology, University Clinical Center in Niš, Serbia, according to standard procedure. All patients were from the same geographical area. Patients with incomplete clinical data were excluded.

Value of SA was determined using standard diagnostic procedures with automated spectrophotometric method, before the start of treatment. According to the value of pretreatment SA, the patients were divided into 2 groups: SA > 30 g/l and ≤ 30 g/l. Clinical stages of disease were determined by Ann Arbor classification. According to their age, the patients were divided into 2 groups: ≤ 60 years and > 60 years. The histological sections were processed by standard techniques, and stained with hematoxylin and eosin (HE) at the Center of Pathology, University Clinical Center Niš, Serbia. Inclusion criteria consist of de novo CD20 + DLBCL.

We analyzed the correlation of SA value with clinical stage and age, as well as the survival of patients with DLBCL. Survival is calculated from the time of diagnosis (OS was defined as date of diagnosis to date of death or date of last contact for those censored), because the date of initiation of therapy in some cases was not known.

Statistical analysis

Comparison of the frequency of distribution of certain modalities of attributive characteristics between groups was performed by Pearson χ^2 test or Fisher exact test. The relationship of certain characteristics was measured by correlation analysis using Spearman's correlation coefficient. OS were estimated using the Kaplan-Meier method. The log-rank test was used in order to determine the difference in survival prognostic factor. The relationship between predictive value of the test variable (the SA value) and the dependent variable (treatment protocol) was established in the logistic regression analysis. Statistical analysis was performed using standard data processing programs and software package SPSS version 18.0.

The values < 0.05 were considered statistically significant

Results

The difference in the frequency of attributive characteristics between the groups (age, clinical stage of disease, the value of SA), was compared. The groups were homogenous in terms of all clinical prognostic characteristics (Table 1).

No significant statistical correlation between the values of serum albumin with age (p = 0.630), clinical stage (p = 0.943) and survival (p = 0.638) in patients with DLBCL treated with CHOP protocol, was found (Table 2).

Table 1. Comparative characteristics of groups

Variable	group CHOP		group R-CHOP		test
Age	N	%	N	%	
< 60 y.	5	35.7	27	49.1	χ^2 = 0.803 p = 0.370
> 60 y.	9	64.3	28	50.9	OR = 0.576
					CI (0.171 - 1.940)
Cl. stage	N	%	N	%	
1	1	7.1	4	7.3	χ^2 = 2.757 p = 0.431
2	4	28.6	23	41.8	
3	7	50.0	15	27.3	
4	2	14.3	13	23.6	
SA	N	%	N	%	χ^2 = 1.558 p = 0.208
> 30 g/l	10	71.4	29	52.7	Fisher exact T = 0.242
≤ 30 g/l	4	28.6	26	47.3	OR = 0.446
					CI (0.125 - 1.596)

Table 2. Correlation analysis of CHOP group

			Age	Cl. stage	Survival
Spearman's	SA	Correlation Coefficient	0.141	0.021	0.138
		Sig. (2-tailed)	0.630	0.943	0.638

Table 3. Correlation analysis of R-CHOP group

			Age	Cl.stage	Survival
Spearman's	SA	Correlation Coefficient	0.201	0.141	0.422
		Sig. (2-tailed)	0.141	0.305	0.001

Significant correlation between serum albumin levels with survival (p = 0.001) in patients with DLBCL treated with R-CHOP protocol was found. There was no difference in the levels of SA in respect of age (p = 0.141) or clinical stage of disease (p = 0.305) (Table 3).

There was no difference in survival compared to the value of SA ≤ 30 g/l vs. > 30 g/l in patients with DLBCL treated with CHOP protocol. Log-rank (Mantel-Cox) = 0.782 (Figure 1).

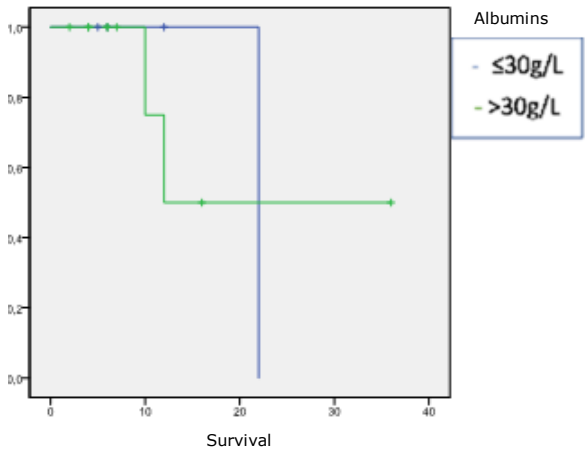

Figure 1. Survival in the CHOP group according to SA values

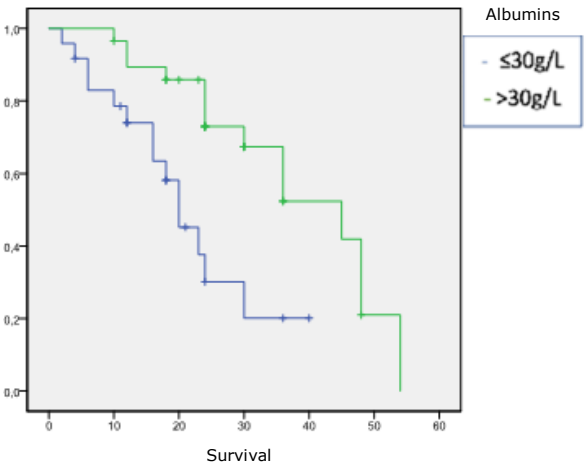

Figure 2. Survival in the R-CHOP group according to SA values

There was statistically significant difference in survival compared to the value of SA ≤ 30 g/l vs. > 30 g/l in patients with DLBCL treated with R-CHOP protocol. Log-rank (Mantel-Cox) = 0.002 (Figure 2). The relationship between the predictive value of SA and the treatment protocol for patients with DLBCL evaluated by logistic regression analysis showed that the level of SA was not a significant predictor of treatment choice (Wald = 1.540, p > 0.05) (Table 4).

Table 4. Logistic regression analysis of SA according to the therapeutic approach of DLBCL Patients

Omnibus Tests of Model Coefficients		Sig.		-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
	1.643	0.200		67.963[a]	0.024	0.037
Variable	B	S.E.	Wald	Sig.	Exp(B)	95% C.I.
SA	0.807	0.650	1.540	0.215	2.241	0.627

Discussion

The results of this study identified SA as a significant predictor of survival in patients treated with the R-CHOP protocol

The connection of hypoalbuminemia and poor prognosis in patients with malignant diseases, is well known (1, 3-7). In 2014, Eatrides et al. confirmed that the level of serum albumin in patients with DLBCL is a powerful predictor of risk (8). In a study with a cut off value of 37 g/l, albumins are marked as predictors of overall survival in patients with DLBCL treated with the R-CHOP protocol and which, together with the other clinical parameters can be useful in identifying patients with high risk of relapse.

A low SA is an independent prognostic marker in patients with DLBCL treated with standard R-CHOP. Low albumin was considered as SA level < 37 g/l. The mechanism by which low level of serum albumin has a negative predictive value in relation to the survival of patients with DLBCL is still not clear enough. There are theories that the low levels of serum albumin are associated with an increased inflammatory response in the tumor mass with a poor nutritional status, as well as the increased release of cytokines. Low SA may also be caused by reduced production in hepatocytes due to the release of cytokines from a tumor, such as IL-6, which blocks the production of albumin in the hepatocytes. Increased levels of TNF may also be associated with low levels of serum albumin (9). Elevated level of interleukin 6 in serum, as one of the cytokines, with almost the main role in the inflammatory process is a negative prognostic factor in patients with DLBCL (10-12). Low serum albumin may also be the result of a strong inflammatory response caused by the presence of the tumor mass.

Hypoalbuminemia is a sign of continuous systemic response (13, 14). Pretreatment albumin level is a strong prognostic factor for OS in patients with DLBCL and can discriminate high-risk patients with inferior prognosis (13, 15).

Poor nutritional status is associated with malignancies, poor performance status and co-morbidities, and could cause the inability to apply adequate therapeutic doses of chemotherapy (9).

Low SA levels are the indicator of the nutritional status and very useful prognostic factor (16).

A systematic review of Gupta et al. (2010) (17), emphasizes the general predictive significance of pretreatment SA in all cancers, including NHL. In order to confirm the level of causality of serum albumin and survival in malignant patients, clinical studies should demonstrate that raising albumin levels by means of intravenous infusion or by hyperalimentation could decrease the excess risk of mortality in cancer (17).

The advantage of pretreatment SA level as a prognostic factor in cancer patients is that it is inexpensive, reproducible and powerful.

There are different results in the literature which show that low SA level, elevated LDH and advanced clinical stage of disease in patients with DLBCL treated with CHOP protocol were poor prognostic factors but that in patients treated with R-CHOP protocol, survival was evident only in advanced clinical stage of disease, and male gender. Serum albumin had no predictive value (18).

The level of serum albumin in patients with DLBCL is very intriguing and because of that several authors proposed a new prognostic scores, including the value of serum albumins.

In 2013, Dalia et al. (19) proposed a new prognostic score "Albumin Adjusted IPI (A-IPI)" that counted: the value of SA < 37g/l, increased levels of LDH, ECOG ≥ 2 and clinical stage III-IV disease, in patients with DLBCL treated with R-CHOP protocol. A-IPI score identified three groups of patients: Very good: A-IPI = 0 Good: A-IPI = 1-2, Poor: A-IPI = 3-4. In the analyzed group of patients, the 4-year PFS (progression free survival) and OS (overall survival), were determined by comparing A-IPI and R-IPI (revised IPI). The comparing of these two prognostic scores, in their study, found that A-IPI score could accurately discriminate group of patients, "poor", with the ability to separate the good and "good" and "very good" risk group of patients with DLBCL. In conclusion, they suggested that SA could be a better surrogate for comorbidity and pro-inflammatory condition in DLBCL patients than their age (19).

Kobayashi et al. (2016) (20) proposed the formation of a new prognostic index "Kyoto Prognostic Index (KPI)" for patients with DLBCL in the rituximab era. KPI counted: > LDH, ECOG ≥ 2, SA < 35 g/l, EN events. Based on these parameters they formed 4 risk groups. Results showed that the KPI was highly predictive and sensitive when compared to the R IPI and the NCCN IPI (An enhanced International Prognostic Index). The same authors found that the age > 60 did not significantly affect the survival rate. "Glasgow prognostic score (GPS) and the modified Glasgow prognostic score" (mGPS), in their point system also contain SA (21, 22).

"The international staging system" contains only the value of SA and B2 microglobulin and is used in the prediction of patients with multiple myeloma, proved to be very useful in predicting patients with DLBCL who were treated with R-CHOP protocol. This score was not a significant predictor for patients with DLBCL treated with CHOP protocol (23).

Conclusion

Our research has confirmed a negative predictive value of pretreatment serum albumin levels in patients with DLBCL treated with protocol R-CHOP. Retrospective studies with a larger number of DLBCL patients who were treated with CHOP protocol would give more significant results of the predictor importance of SA. Prognostic indexes, which as part of the point system include the value of the SA, can be very useful in predicting patients with DLBCL.

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PREDIKTORNI ZNAČAJ VREDNOSTI ALBUMINA U SERUMU OBOLELIH OD DIFUZNOG B KRUPNOĆELIJSKOG LIMFOMA U PRERITUKSIMAB I RITUKSIMAB ERI

Ljiljana Tadić[1], Nikola Krstić[2]

[1]Vojna bolnica Niš, Odelenje za interne bolesti, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija

Kontakt: Ljiljana Tadić
Bulevar dr Zorana Đinđića 66, 18000 Niš, Srbija
E-mail: mitanik19@gmail.com

Difuzni B krupnoćelijski limfom (*DLBCL*), predstavlja najprisutniju podgrupu ne-Hočkinskih limfoma. Cilj autora je da provere postojanje značaja vrednosti albumina u serumu pre početka lečenja, kao nezavisnog faktora prognoze bolesti, na prostoru jugoistočne Srbije, u prerituksimab i rituksimab eri.

U studiju je bilo uključeno ukupno 55 bolesnika sa *DLBCL* (*R-CHOP* grupa) i 14 bolesnika lečenih po *CHOP* protokolu (*CHOP* grupa). Bolesnici su podeljeni u dve grupe prema vrednostima SA: SA > 30 g/l i SA ≤ 30 g/l. Analizirana je korelacija vrednosti SA sa kliničkim stadijumom bolesti i godinama života, kao i preživljavanje obolelih od *DLBCL* u odnosu na vrednosti SA, prema terapijskom protokolu kojim su lečeni.

Nije bilo značajne statističke korelacije vrednosti SA sa godinama života (p = 0,630), kliničkim stadijumom bolesti (p = 0,943) i preživljavanjem (p = 0,638) kod obolelih od *DLBCL* lečenih po protokolu *CHOP*. Kod ispitanika u grupi *R-CHOP*, postoji značajna korelacija vrednosti SA sa preživljavanjem (p = 0,001), bez značajne korelacije vrednosti SA sa godinama starosti (p = 0,141) i kliničkim stadijumom bolesti (p = 0,305). Nije bilo značajne razlike u preživljavanju u poređenju sa vrednostima SA ispitanika iz *CHOP* grupe/Log-rank = 0,782. Postojala je značajna razlika u preživljavanju u poređenju sa vrednostima SA ispitanika u *R-CHOP* grupi/Log-rank = 0,002.

Odnos između prediktivne vrednosti SA i protokola lečenja *DLBCL*, procenjen logističkom regresionom analizom, pokazao je to da nivo SA nije značajan prediktor za izbor lečenja (Wald = 1.540, p > 0.05).

Naše istraživanje potvrdilo je negativan prediktorni značaj smanjenog nivoa SA pre početka lečenja, kod obolelih od *DLBCL*, lečenih po terapijskom protokolu *R-CHOP*. Retrospektivne studije sa većim brojem *DLBCL* obolelih, lečenih po protokolu *CHOP*, dalo bi značajnije rezultate o prediktornom značaju SA za ovaj terapijski modalitet. Prognostički indeksi, koji kao deo bodovnog sistema sadrže i vrednost SA, mogu biti vrlo korisni u predikciji obolelih od *DLBCL*.

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Ključne reči: *DLBCL, albumin, R-CHOP, CHOP*

Review article

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IMPLICATIONS OF OXIDATIVE STRESS IN END-STAGE KIDNEY DISEASE PATIENTS: A REVIEW OF CAUSATIVE MECHANISMS, CURRENT CONCEPTS

Vassilios Liakopoulos[1,2], Garyfallia Varouktsi[1,2], Ariti Tsinari[1,2], Andrej Veljković[3], Gordana Lazarević[4,5], Zoran Perišić[4,5], Jovan Hadži-Djokić[6], Gordana Kocić[3], Stefanos Roumeliotis[1,2]

Oxidative stress (OS), termed as the imbalance between antioxidants and pro-oxidants in favor of the latter, is highly prevalent in chronic kidney disease (CKD) even at early stages and is gradually increased, in parallel to deterioration of kidney function. In end-stage kidney disease (ESKD), OS is further aggravated and associated with various adverse outcomes, including atherosclerosis and cardiovascular disease. In this review, we aim to present the clinical implications of OS, the pathogenetic causative mechanisms and the potential therapeutic interventions in both hemodialysis and peritoneal dialysis patients.

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Key words: *cardiovascular disease, chronic kidney disease, end-stage kidney disease, hemodialysis, oxidative stress, peritoneal dialysis, vitamin C, vitamin E*

[1]Division of Nephrology and Hypertension, 1st Department of Internal Medicine, AHEPA Hospital, Thessaloniki, Greece
[2]Aristotle University of Thessaloniki, School of Medicine, Thessaloniki, Greece
[3]University of Niš, Faculty of Medicine, Department of Biochemistry, Niš, Serbia
[4]University Clinical Center Niš, Division of Cardiology, Niš, Serbia
[5]University of Niš, Faculty of Medicine, Niš, Serbia
[6]Serbian Academy of Sciences and Arts, Belgrade, Serbia

Contact: Vassilios Liakopoulos
1 St. Kyriakidi Street, 54636 Thessaloniki, Greece
E-mail: vliak@auth.gr

Introduction

Chronic kidney disease (CKD) is a major health problem worldwide, affecting 11-13% of the global population, a percentage that is expected to rise dramatically in the next decade (1). Cardiovascular (CV) disease is highly prevalent and remains the major cause of mortality in these patients. Even at early CKD stages 1-2, patients are at risk for sudden CV death and as the disease progresses to end stage kidney disease (ESKD), this risk is further exacerbated (2). Thus, when compared to the general population and after adjustment for age, diabetes and hypertension, ESKD patients have a 2-fold increased risk for CV death (3, 4). This is why the expected remaining lifespan of ESKD patients undergoing maintenance hemodialysis (HD) is 8 years for patients between 40 and 44 years old and under 4.5 years for 60-64 years old (4). Although the Framingham traditional CV risk factors such as age, gender, obesity, hypertension, smoking, diabetes, dyslipidemia, sedentary lifestyle and heart failure are over-represented, they cannot solely explain the heavy CV burden seen in these patients. During the past decades, novel-uremia-specific non-traditional risk factors have emerged, including anemia, inflammation, oxidative stress (OS), endothelial dysfunction, hypoalbuminemia, malnutrition and abnormal calcium metabolism. These novel risk factors might explain the CV burden of ESKD patients and represent potential treatment targets. OS, a novel non-traditional risk factor, is a common pathogenetic mechanism underlying both the occurrence and progression of CV disease in ESKD patients.

Definition and markers of OS in CKD and ESKD

Every molecule that is missing an electron is defined as "pro-oxidant", whereas an agent that donates electrons to other molecules is termed "anti-oxidant". OS is the disturbance of balance between pro-oxidants and anti-oxidant agents, in favor of pro-oxidants. Pro-oxidant molecules, such as nitric oxide (NO) and reactive oxygen species (ROS) are highly reactive agents and in order to become stabilized, they "steal" an electron from adjacent biomolecules (including proteins, lipids, nucleic acids and carbohydrates) causing structural and functional

modification and subsequent oxidative injury in the cells and tissues. In an attempt to counteract the injury caused by free radicals, endogenous antioxidants are generated to de-activate ROS. Since free radicals are chemically unstable molecules, their measurement in plasma or serum is very difficult to be performed. Therefore, the serum or plasma levels of oxidative molecules and endogenous antioxidants might serve as markers of OS status. Markers of protein oxidation include thiols, carbonyls, homocysteine, myeloperoxidase (MPO), oxidized albumin asymmetric dimethylarginine (ADMA) and advanced oxidation proteins products (AOPPs); markers of nucleic acid oxidation include 8-hydroxy deoxyguanosine and 8-Oxo-2-deoxyguanosine; markers of lipid peroxidation include malondialdehyde (MDA), oxidized low density lipoprotein (ox-LDL), F2-isoprostanes, thiobarbituric acid reactive substances (TBARS), lipoperoxides, advanced lipid oxidation products and advanced glycation end-products (AGEs) are the main marker of carbohydrate oxidation. On the other hand, enzymatic antioxidant markers include catalase (CAT), superoxide dismutase (SOD), glutathione transferase, glutathione peroxidase and total antioxidant capacity (TAC), whereas non-enzymatic antioxidant markers include glutathione, reduced glutathione, uric acid (5), albumin, transferrin and ferritin, zinc, copper and selenium, N-acetylcysteine (NAC), vitamins B, C, D, E, A-lipoic acid, flavonoids, polyphenols, L-carnitine, Q-enzyme 10, green tea, curcumin, statins and omega-3 fatty acids (6).

In early CKD, OS is present and increases gradually with kidney function impairment to ESKD (7). This could be attributed to various reasons. First, due to the uremic environment, several molecular pathways - such as the mitochondrial respiratory chain reaction, the activity of dinucleotide phosphate oxidase (NADPH) and NO synthase- are severely de-arranged and lead to overproduction of pro-oxidants (8). Second, due to inadequate kidney clearance, these pro-oxidants accumulate in the circulation and third, the naturally occurring antioxidants present reduced activity and decreased concentrations in plasma and serum (9). Patients even at very early CKD stages have higher OS status compared to healthy subjects (10), whereas in a cohort of 87 patients at CKD stages 1-4, it was shown that F2-isoprostanes increased and TAC decreased progressively, across the stages of the disease (11).

Compared to pre-dialysis CKD, in ESKD, OS is further exacerbated and results in oxidative damage of the kidney (renal ischemia, glomerular damage and tubular apoptosis), upregulation and activation of systemic inflammatory cytokines, such as c-reactive protein (CRP), interleukin-6 and -10 (IL-6, IL-10), tumor necrosis factor-alpha (TNF-α) and subsequently endothelial dysfunction and atherosclerosis (12, 13). "Although dialysis per se triggers all the aforementioned molecular pathways, it was not clear whether the main culprit for the increased OS seen in ESKD patients is dialysis procedure or kidney damage." Data from studies where ESKD patients undergoing HD were subjected to kidney transplantation and kidney function was restored, showed that within 2 months post-transplantation all markers of OS and inflammation were normalized (14).

OS in ESKD patients undergoing maintenance HD

Compared to pre-dialysis CKD patients and healthy subjects, ESKD patients undergoing maintenance HD present significantly increased OS status for various reasons. First, the dietary instructions in ESKD to avoid consumption of fruits and vegetables (that are rich in potassium) and the possible malnutrition that usually accompanies these patients might result in decreased intake of important antioxidants, such as vitamins B, C, D and E (15, 16). Second, various comorbidities that are common among ESKD patients, including hypertension, anemia, advanced age, dyslipidemia, diabetes mellitus, and atherosclerosis trigger generation of free radicals (17). Third, in ESKD, the endogenous antioxidant defense systems are significantly impaired (18) and four, the chronic micro-inflammation status that usually accompanies HD, might also contribute to generation of pro-oxidants. Finally, the HD procedure per se triggers OS through several pathogenetic mechanisms.

Due to the contact of blood with the bioincompatible, artificial membranes and dialysate, within 15 minutes after initiation of dialysis, ROS are abruptly generated and released to circulation by white blood cells (19, 20). It is estimated that after every 4 hour HD session ROS levels are fourteen times higher than before the session (21), whereas certain amounts of antioxidant vitamins and trace elements are lost (22, 23). Since the main mechanism underlying the oxidative burst of polymorphonuclear white blood cells is the bioincompatible dialyzer membranes, several investigators aimed to study whether the development of new, more biocompatible membranes might ameliorate OS and benefit HD patients. Several studies reported that compared to the older, cuprophane membranes, newer, more biocompatible polysulfone membranes were associated with decreased lipid peroxidation status (24, 25), reduced generation of ROS (26, 27) and increased levels of the antioxidants CAT (25) and vitamin E (28). Similarly, compared to cuprophane, regenerated cellulose membranes were related with reduced production of free radicals (29). Of note, no study has assessed the effect of these newer, more biocompatible membranes with clinical hard CV outcomes. Moreover, although the newer, synthetic, more biocompatible membranes are better compared to the old, cuprophane membranes, these membranes still trigger activation of pro-oxidants and therefore their designation as "biocompatible" is a debatable term.

Another HD-related factor implicated in the pathogenesis of OS is the use of anticoagulation; heparin is suggested to trigger oxidation of fatty acids (30), whereas citrate anticoagulation might be of benefit, regarding lipid peroxidation status (31). In a comparative study, Gritters et al. (32) examined the white blood cells' oxidative burst during HD session using citrate or classical heparin or low molecule weight dalteparin. The authors found that

in HD with classical or low molecular weight heparin, ROS were abruptly increased within the first ten times of HD session, whereas HD with citrate was associated with significantly decreased markers of lipid peroxidation, such as MPO and ox-LDL.

Ultrapure dialysate is fundamental in HD. Even the slightest concentration of bacterial toxins in the dialysate compromises the biocompatibility of HD session and triggers the activity of pro-oxidants (33, 34). On the other hand, HD treatment with ultrapure dialysate is suggested to ameliorate both inflammation and OS biomarkers and improve anemia status (35-37). A large meta-analysis of 31 studies including 1580 HD patients clearly showed that HD with ultrapure dialysate successfully suppressed plasma levels of ox-LDL, MPO, IL-6 and MPO (38).

Another factor related with increased OS in HD is problematic or failed HD vascular access, such as the use of misplaced central venous catheters and malfunctioning arteriovenous fistulae or grafts (39, 40). Weiss et al. (39), reported that in arteriovenous fistulae and grafts that were removed due to thrombotic episodes or aneurysm formation, markers of lipid, carbohydrate and protein oxidation were overexpressed. Of note, the majority of these tissues did not present inflammation.

Accumulating evidence suggests that anemia triggers OS in HD patients (41-43), whereas its correction significantly abrogates the oxidation process (42, 44, 45). Besides erythropoietin stimulating agents, the treatment of anemia in HD also includes intravenous iron infusion. Intravenous infusion of 100 mg iron sucrose triggered an abrupt formation of MDA 30 minutes after the initiation of the process in pre-dialysis anemic CKD patients (46, 47), and generation of MDA and OS-derived DNA damage in HD patients (48-50). To explore the degree of OS caused by intravenous iron administration, Tovbin et al. (51), infused 100 mg of iron saccharate for 3.5 hours in 19 HD patients and found a 37% increase in markers of protein oxidation after the end of HD session. The duration of iron infusion influenced the oxidative response triggered. Rapid or bolus intravenous administration caused an abrupt increase in free radicals, probably due to transient oversaturation of transferrin and iron overload (52, 53). Slow intravenous infusion of iron agents is suggested in HD patients, to give time to naturally occurring antioxidant defense mechanisms to counteract and neutralize the gradual generation of free radicals (54, 55).

OS in ESKD patients on peritoneal dialysis

Not all ESKD patients undergo HD treatment; it is estimated that nearly 196,000 ESKD patients worldwide, representing 12–15% of the dialysis population choose peritoneal dialysis (PD) as renal replacement therapy (RRT) (56). Although in HD, OS has been thoroughly investigated, in PD, the data remain limited. Compared to pre-dialysis CKD and ESKD patients, OS is higher in PD patients; however, the OS in PD is triggered without using bioincompatible membranes, dialysate, malfunctioning vascular access or heparin. The main culprit that is responsible for the OS in PD patients is the biocompatibility of PD solutions, including the high glucose concentration, the acidic pH, the lactate buffer and the increased osmolarity (57). Peritoneal mesothelial cells exposed to conventional PD solutions die from apoptosis within 40 minutes from their exposure (58). PD solutions include water, high glucose, lactate buffer and various electrolytes. When these solutions are heated, before entering the peritoneal cavity, AGEs and glucose degradation products (GDPs) are formatted (59). Exposure of peritoneal mesothelial and endothelial cells to the high glucose and the AGEs of the PD solution activate several molecular pathways similar to those underlying the pathogenesis of diabetic CKD, including the polyol pathway, glucose-oxidation and diacylglycerol pathway and activation of protein kinase C (60). Subsequently, growth factors (such as vascular endothelial growth factor), inflammatory (such as IL-6, IL-1, IL-10 and CRP) and OS markers are activated in the peritoneal cavity. Chronic exposure of the peritoneal cavity to these factors results in peritoneal fibrosis and thickening, angiogenesis, vasculopathy, progressive transformation of the peritoneal membrane and systemic OS and inflammation (60).

Therapeutic options to ameliorate OS in HD and PD

To counteract the deleterious effects of OS in HD, several lifestyle modifications have been proposed, including cessation of smoking, optimal management of blood pressure, lipids and glycemic control. Other interventions include use of a functional vascular access, correction of anemia status, slow intravenous infusion of iron and use of biocompatible dialyzers and ultrapure dialysate. Similarly, in PD, a multitargeted approach is needed, including limited salt and water intake, strict glycemic control and use of newer, more biocompatible PD solutions with low glucose, low GDPs, bicarbonate lactate and neutral pH.

Moreover, several investigators suggested that supplementation with exogenous antioxidants, including vitamin C, vitamin E and NAC could benefit EKSD patients. It has been shown that in every dialysis session about 66 mg of vitamin C are lost through the dialyzer, whereas vitamin C depletion is highly prevalent in HD patients (61). Several studies examined whether oral or intravenous supplementation of vitamin C might ameliorate OS in HD patients and produced contraindicatory results (62-66). Moreover, these studies have major limitations, including small sample size and wide diversity on follow-up time and doses used. A recent meta-analysis of 11 randomized controlled trials and 491 HD patients (67) reported that vitamin E supplementation improved endothelial dysfunction, and significantly decreased CRP and MDA levels, compared to the control group. Since vitamin E is a powerful antioxidant with anti-inflammatory properties and the main mechanism of OS in HD is the exposure of blood to the artificial dialyzer, coating the inner surface of HD membranes with vitamin E was an attractive approach to ameliorate OS. Data

from meta-analyses have coherently showed that HD with vitamin E coated membranes significantly decreased MDA and TBARS (68), improved anemia and decreased both inflammation (69) and OS status (70).

In PD, the most promising antioxidant that has been studied is NAC, a ROS scavenger that might decrease accumulation of AGEs and inflammatory cytokines in the peritoneal cavity (6, 18, 71). Two studies in PD cohorts independently reported that oral NAC supplementation decreased OS, suppressed inflammation, preserved residual renal function and protected the peritoneal membrane from OS-derived sclerosis and injury (72, 73).

Conclusion

OS is highly prevalent even at early stages of CKD and gradually increases with deterioration of kidney function. In ESKD, OS is further aggravated and is associated with adverse events, including CV disease and mortality. In HD patients, the main pathogenetic mechanism underlying the generation of free radicals is the contact of blood with the artificial, synthetic, bioincompatible dialyzer membranes and dialysate. PD patients suffer from lesser degree of OS compared to HD patients, mainly due to the composition of PD fluids. To date, there are no specific guidelines regarding the supplementation of antioxidant agents to ameliorate the increased OS in HD or PD patients. Future, large, randomized, placebo-controlled trials are required to draw definite conclusions.

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IMPLIKACIJE OKSIDATIVNOG STRESA KOD BOLESNIKA U ZAVRŠNOJ FAZI HRONIČNE BOLESTI BUBREGA: PREGLED KAUZATIVNIH MEHANIZAMA I TRENUTNIH KONCEPATA

Vassilios Liakopoulos[1,2], Garyfallia Varouktsi[1,2], Ariti Tsinari[1,2], Andrej Veljković[3], Gordana Lazarević[4,5], Zoran Perišić[4,5], Jovan Hadži-Đokić[6], Gordana Kocić[3], Stefanos Roumeliotis[1,2]

[1]Odeljenje za nefrologiju i hipertenziju, Prvi departman interne medicine, AHEPA bolnica, Solun, Grčka
[2]Aristotelov univerzitet u Solunu, Medicinski fakultet, Solun, Grčka
[3]Univerzitet u Nišu, Medicinski fakultet, Katedra za biohemiju, Niš, Srbija
[4]Univerzitetski klinički centar Niš, Klinika za kardiologiju, Niš, Srbija
[5]Univerzitet u Nišu, Medicinski fakultet, Katedra Interna medicina i zdravstvena nega, Niš, Srbija
[6]Srpska akademija nauka i umetnosti, Beograd, Srbija

Kontakt: Vassilios Liakopoulos
Kyriakidi 1, 54636 Solun, Grčka
E-mail: vliak@auth.gr

Oksidativni stres (OS), označen kao disbalans između antioksidanasa i prooksidanasa, u korist potonjih, ima izraženu prevalenciju kod hronične bolesti bubrega (HBB), čak i u ranim fazama bolesti i postepeno se povećava, paralelno sa deterioracijom bubrega. U završnoj fazi hronične bolesti bubrega, OS se dalje pogoršava i povezuje se različitim nepovoljnim ishodima, uključujući aterosklerozu i kardiovaskularne bolesti. U ovom pregledu, cilj je da predstavimo kliničke implikacije OS, patogenetske kauzativne mehanizme i potencijalne terapeutske intervencije, kod bolesnika na hemodijalizi i peritonealnoj dijalizi.

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Ključne reči: *kardiovaskularne bolesti, hronična bolest bubrega, završna faza hronične bolesti bubrega, hemodijaliza, oksidativni stres, peritonealna dijaliza, vitamin C, vitamin E*

Review article

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BLUE MOONLIGHTING IN THE IMMUNE RESPONSE: ROLES OF COPPER AND CERULOPLASMIN IN THE PATHOGENESIS OF INFLAMMATION AND IMMUNE-MEDIATED DISEASES

Jelena Milenković[1], Branka Djordjević[2], Dijana Stojanović[1], Olivera Dunjić[1], Vanja Petrovski[3]

Increase in serum copper levels and/or its main blood carrier - ceruloplasmin (Cp) is a constant finding in some human diseases. One of the best-known roles of Cp is the regulation of cellular iron efflux in situations of hypoxia. Nevertheless, copper and Cp are involved in multiple physiological processes, such as redox balance, regulation of transcription factors, neuronal growth, some immune functions: microbicidal activity, cytoprotective barrier, lymphocyte proliferation, etc. Ceruloplasmin is an acute phase reactant and therefore its levels increase in conditions of acute infections or inflammation. Also, copper dyshomeostasis has been clearly established in many inflammatory autoimmune diseases, malignancies, neurological and obstetric disorders. Changes of copper and Cp metabolism are reported in the pathogenesis of diabetes mellitus and cardiovascular diseases. Besides, alterations of serum copper can be utilized as a prognostic and predictive biomarkers. However, interpretation of these data is not fully recognized in the routine clinical practice. Therefore, the aim of our work is to review current knowledge and recent evidence about the roles of copper and Cp as a part of the immune response in the etiopathogenesis of multiple diseases and present the usefullness of interpretation of their altered levels.

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Key words: *inflammation, macrophages, lymphocytes, iron homeostasis, trace elements*

[1]University of Niš, Faculty of Medicine, Department of Pathophysiology, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Department of Biochemistry, Niš, Serbia
[3]Health Center in Niš, Niš, Serbia

Contact: Jelena Milenković
81 Dr Zorana Djindjića Blvd., 18000 Niš, Serbia
E-mail: jelena.milenkovic@medfak.ni.ac.rs,
jelenaradovic982@gmail.com

Introduction

Increased levels of copper (Cu) in the blood or hypercupremia is a constant finding in certain human diseases, as well as some physiological states, which is often underestimated and left out from interpretation. However, assessment of serum copper levels (SCL), or its main blood carrier - ceruloplasmin (Cp), may help in guiding a diagnostic protocol.

Ceruloplasmin and copper are involved in multiple physiological processes, cellular mechanisms, and signaling pathways. Ceruloplasmin has been called a promiscuous or moonlighting enzyme, mainly because there is a multitude of substrates it can act on. Moreover, copper may bind to the sites on Cp other than the active site, creating additional accessory functions. One of the best-known Cp functions is ferroxidase activity by which it helps iron efflux from the cells in situations of hypoxia. It also performs oxidation of biological amines, lipids, and oxidative inactivation of NO. Together with zinc and selenium, copper displays an important role in the immune system. Human macrophages use copper as a bactericidal and fungicidal agent in phagosomes, which is achieved by its marked accumulation during cell activation. Fluctuations in the copper concentration influence the activity of a transcription factor NF-κB, as well as hypoxia-inducible factor 1 alpha (HIF-1α). Copper is a catalytic cofactor of the two important anti-oxidative enzymes, cytosolic and extracellular Cu(II)/Zn(II)-superoxide dismutases (SOD1 and SOD3). Besides, copper is needed for axonal outgrowth, synapse integrity, neuronal activation, and motor neuron maintenance (1-8).

Nevertheless, copper may be potentially toxic and cause dysregulation of physiological processes. Its involvement in oxidation-reduction reactions requires close regulation because through the shift of its redox states it can generate damaging hydroxyl radical and interfere with the function of other intracellular metals. It may displace other metals from its cognate organic ligands, leading to inappropriate

changes in protein functions. This process is explained by the Irving-Williams series of the relative stabilities of complexes formed by divalent first-row transition metal ions, which increase across the period to maximum stability at copper (1, 3, 7).

Physiologically, females have slightly higher copper levels than men, which additionally increase during pregnancy. Reactive or secondary changes in the concentration or availability of copper mostly occur in immunological and inflammatory conditions. Also, total copper serum concentrations frequently increase in cancer patients. Several studies estimated its values in the diagnostics and staging, as well as a prognostic marker in various diseases (7, 9, 10).

The objective of this scoping review was to summarize current knowledge and recent evidence about the roles of copper and Cp as a part of the immune response in the etiopathogenesis of multiple diseases that have an immune or inflammatory background. We also tried to present the usefulness in the interpretation of their altered serum levels in diagnostics.

Basic physiological concepts of copper metabolism

Copper is an essential trace element (oligoelement), with many natural sources and diverse metabolic functions. It participates in many biochemical reactions and structures of many biomolecules. Copper ions can exist in a reduced (Cu+) or an oxidized (Cu2+) state. In the reduced form, it is mainly found in intracellular space, as oxidized in fluids, and in redox enzymes it shuttles between the two forms. This gives copper an affinity for binding to the different side-chains of amino acids and thus provides the ability to interact with many proteins. Therefore, it is a part of proteins such as transcription factors, transporters, chaperones and storage proteins, oxidases, monooxygenases and oxidoreductases (ascorbate oxidase, dopamine-monooxygenase, lysyl oxidase, phenylalanine hydrolase, tyrosinase, *e.g.*), electron transfer proteins (cytochrome c oxidase), free radical scavengers (Cu, Zn superoxide dismutase - SOD), and others (1-3, 7).

Copper is transported across the apical membrane of enterocytes by a copper transport protein 1 (Ctr1), a redundant high-affinity copper cellular transporter, and can transfer via a divalent metal ion transporter 1 (DMT1). Then it becomes transferred by the Atox1 Cu chaperone to the ATP7A enzyme (a Cu-transporting P-type ATPase) at the basolateral membrane into the portal circulation and liver. It is stored in the liver, mainly in the mitochondria, specifically in the complex IV of the respiratory chain. When in excess, it can be sequestrated by metallothioneins or glutathione. ATP7A also participates in copper transport across different polarized cell layers, as well as intracellular organelles such as Golgi apparatus and phagosomes. Another ATPase ATP7B is a transmembrane copper transporter specific for the liver, which aids in copper loading on apo-ceruloplasmin and export of its excess into the bile (1-4, 7). From a clinical point of view it is important to say that it takes a few weeks for Cp to reflect changes of copper in diet (11).

Ceruloplasmin is a multifunctional protein that tightly binds 6 atoms of copper per molecule. It is recognized as a carrier of most copper in the blood (about 40-70%), which it transports from the liver to the distal tissues. A smaller percent of copper is delivered by albumin, α2-macroglobulin (transcuprein), and several other small molecules. Ceruloplasmin belongs to the α2-glycoprotein fraction and has two molecular Cp isoforms: secreted and a membrane glycosylphosphatidylinositol (GPI)-anchored form. Secreted Cp is a plasma oxidase, primarily known for its potent Cu-based ferroxidase activity, which makes it essential for iron homeostasis. In the presence of O2 Cp may convert iron from ferrous (Fe2+) to ferric ion (Fe3+), therefore assisting and enhancing iron efflux from cells' storage through the ferroportin and iron-binding to plasma apotransferrin or apoferritin. The iron release from macrophages is specifically induced under conditions of hypoxia, to be used for erythropoiesis (10-12). The Cp gene contains a response element for the alpha subunit of hypoxia-inducible factor (HIF1-alpha) transcription factor, the main regulator of cellular response to hypoxia. In this context, Cp is thought to be reserved for iron regulation due to relative hypoxia. Besides hypoxia and apotransferrin, the process requires an available intracellular "labile iron pool" and a low extracellular free iron level, which creates a negative iron gradient. Ferroportin is the only known export pathway of intracellular non-heme-associated iron (10, 13).

Monocytes and macrophages additionally display the GPI-membrane-linked Cp in lipid rafts, with increased expression and colocalization with ferroportin after iron treatment. This GPI-anchored form is encoded by an alternatively spliced variant of the Cp gene (12-15). Another multicopper protein homologous to Cp is hephaestin. However, it is a transmembrane ferroxidase mostly found in enterocytes of the villi and is responsible for transporting dietary iron (16). Besides, a few more molecules are determined to bind and use copper, such as zyclopen, native prion protein (PrPC), clotting factors V and VIII, etc (4, 10, 17).

However, all physiological roles of Cp are still not well defined. Ceruloplasmin is also an acute-phase reactant, meaning that its levels increase during inflammation, infection, malignancies or trauma. Due to the expression of ATP7B copper transporter, lymphocytes and macrophages are not likely to accumulate copper (18). However, ATP7A protein levels rise with macrophage (M1) activation and it translocates to the phagosomes, where increases copper content aiming for the preparation for antimicrobicidal functions. Concurrently, there is an increase in Cp expression (1).

Ceruloplasmin is involved in the regulation of redox reactions, but its roles are still not defined well, because depending on a situation it was determined both anti-oxidant and pro-oxidant.

Ceruloplasmin is considered the main extracellular radical scavenger that inhibits a variety of oxidative reactions, such as superoxide dismutation, peroxidation, prevents spontaneous oxidation of

Fe2+ (the Fenton reaction), as well as consequent damage to the biomolecules. It also performs oxidative inactivation of NO and some biogenic amines. Ceruloplasmin oxidates NO to NO+ with subsequent hydration to nitrite, its bioactive reservoir (5, 10, 19, 20).

Average SCL for adults is estimated as from 11 up to 22 μmol/L (70-140 μg/dL), with slightly higher levels in females - 12.5-24 μmol/L (80-155 μg/dL). Infants from birth to the 6th month have copper levels between 3-11 μmol/L, up to 6 years of 14-30 μmol/L, after which they decline to 13-25 μmol/L. Normal values of copper in 24h urine are < 60 μg/24h (1.0 μmol/24h) in adults, while significantly higher levels are present in Wilson disease, that is > 200 μg/24h urine (> 3.0 μmol/24h). Average Cp serum levels are 20-60 mg/dL (0.93 to 2.65 μmol/L). Besides, many medications are known to increase Cp levels, e.g. anticonvulsants (2, 10, 21-23).

Primary disorders of copper and Cp metabolism

Inherited disorders of copper homeostasis are rare and commonly affect Cp or other proteins involved in the copper transfer and/or excretion system. They are characterized by copper accumulation in specific organs which, however, creates its deficiency in the remaining parts of the body. Mutations in the gene of Cp may cause aceruloplasminemia with resulting iron accumulation and related tissue damage. Menkes diseases and Wilson disease (WD), or hepato-lenticular degeneration, are genetic disorders with mutations in the genes for ATP7A and ATP7B, respectively. The mildest form of Menkes disease is occipital horn syndrome. Disruption in the ATP7A gene leads to the copper sequestration into the intestine and kidneys, while ATP7B defect causes deficiency in biliary copper excretion and its accumulation, dominantly in the liver and brain (basal ganglia), with their progressive damage (1, 8, 24-26). Signs and symptoms are related to the deficiency in enzymes that require copper for its function, such as lysyl oxidase and dopamine-β-hydroxylase. These include connective tissue abnormalities, neurodegeneration, dysautonomia, ataxia, dystonia, psychiatric disorders, liver damage, amenorrhea and subfertility, anemia, etc. (8, 24-28).

These conditions are usually associated with lower than normal Cp blood levels (< 20 mg/L). Therefore, diagnostic criteria for WD are low serum Cp and copper levels together with a high urine copper excretion. It is important to start copper chelation therapy, along with zinc substitution, early in the onset of WD, because the therapy is ineffective in advanced stages. There are ongoing studies over genetic therapy for Menkes disease, because chelation therapy is ineffective in this case and the disease is lethal (1, 26, 27).

In a mouse model of WD, several important observations were made regarding copper handling in hepatocytes. Copper availability within a cell must be carefully controlled to avoid damaging copper-dependent redox cycling. One of the first responses to the copper overload was a downregulation of Ctr1, Cu-influx protein, and potential engagement of copper-export mechanisms. This was followed by a dynamic change in cellular metabolism with the formation of intracellular and extracellular copper deposits and enhanced accumulation in surrounding cells, especially lymphocytes and macrophages. It is important to note that although copper levels increased in the immune cells, this redirection seemed not to cause pathological copper accumulation or disturb cellular metabolism, likely due to the existence of other copper transporting enzymes. Besides, concomitant with the dynamics of copper compartmentalization, specific changes occurred in the metabolic pathways of hepatocytes. Importantly, during all evolution stages, significant retardation of lipid metabolism and oxidative-reductive processes was present (18).

Reactive changes of copper and Cp metabolism

Copper-containing proteins show a range of roles in support of metabolic and homeostatic processes. Therefore, it is not surprising that various pathological derangements can create unbalance in copper distribution. Although hepatocytes are the main producers of Cp, other cell types are capable to produce and secrete Cp as well, such as activated macrophages and mononuclear cells during inflammation, lymphocytes, astrocytes, leptomeningeal cells, choroid plexus of the brain, retina, kidneys, spleen, the epithelial lining of the uterus, placenta (syncytiotrophoblasts), mammary epithelial cells, Sertoli cells, adipocytes, etc. (5, 10, 14, 23, 29, 30). Besides copper itself, the main regulators of Cp expression are hypoxia, inflammatory cytokines, and female sex hormones (estrogens and progestagens). Hence, healthy females have slightly higher SCL and Cp compared to males (10). Regarding their cellular sources, increased copper and Cp plasma levels are commonly expected in inflammation, infections, lymphoproliferative and autoimmune disorders, pregnancy or contraceptive use, hepato-biliary disorders, malignant and some neurologic diseases (10, 30-33).

Copper and Cp in acute inflammation and infection

Increased SCL and Cp levels are common in infection and inflammation, because they are a part of the acute phase response. As an acute phase reactant, Cp serum concentration and activity increase nearly double or approximately up to 900 μg/mL. Accordingly, the plasma levels of copper increase, while the levels and availability of iron decrease, following the change in its regulatory proteins (ferritin, transferrin, hepcidin, etc.). The acute phase response is largely mediated by proinflammatory cytokines (IL-1, IL-6, TNF-α, and IFN-γ) (10, 15, 20, 23). And IL-6 seems to be the main cytokine for stimulation of Cp gene transcription, as there are three IL-6 response elements in the enhancer and promoter regions of its gene. Besides, Cp gene

expression can be induced by bacterial lipopolysaccharide (LPS) during inflammation (10, 34).

Peripheral blood lymphocytes constitutively express both isoforms of Cp (soluble and GPI-linked membrane form). Importantly, the greatest Cp expression was found on natural killer (NK) cells (CD3-CD16+/CD56+ lymphocytes) compared to other major lymphocyte subsets (10, 14, 23). In both infection and inflammation, active proliferation of lymphocytes is accompanied by the increase in serum Cp concentration. There is also speculation that the Cp increase might additionally originate from the direct secretion and/or surface shedding of Cp from peripheral blood leukocytes. Solubilization of GPI-linked proteins from the surface of activated NK cells has been described and was mediated by the actions of matrix metalloproteases (23, 35). Ceruloplasmin surface expression and shedding following lymphocyte activation are hypothesized to provide a part of the cytoprotective barrier by utilizing the Cp antioxidant capacity (10).

As already mentioned, upon activation, macrophages actively accumulate copper into the phagosomes, where it adds to the microbicidal defense (1). And vice versa, the copper deficiency was shown related to decreased respiratory burst and microbicidal activity of phagocytic cells (23). Free copper ions are prone to oxidation and reduction by which they may propagate the formation of reactive oxygen species (ROS) (20). In addition, when stimulated by LPS under inflammatory conditions, Cp potentiates inducible NO synthase (iNOS) activity and production of NO in microglial cells (10).

Moreover, changes in the copper concentration influence the activity of NF-κB and induce Golgi-complex-independent secretion of interleukins and cytokines, such as IL-1 (4).

Ceruloplasmin may cause both anti- and prooxidant effects on polymorphonuclears (PMN). Particularly, it reduced spontaneous and inflammation-induced accumulation of superoxide radicals, but also triggered a rapid increase of intracellular oxidation products (20). Ceruloplasmin acts as a potent endogenous inhibitor of neutrophil myeloperoxidase (MPO), some serine proteases, and forms complexes with other plasma proteins during inflammation, such as lactoferrin (22, 31, 36). Myeloperoxidase is a potent oxidant capable to exacerbate oxidative stress (OxS) in inflammation, while Cp was demonstrated to inhibit MPO production of hypochlorous acid by about 50% (20, 31). However, in the situations of severe inflammation, such as sepsis, Cp is overwhelmed and destructed by hydrogen peroxide and proteases. In addition, tyrosine residues of Cp can be nitrated in OxS by peroxynitrite (generated by MPO) which declines its ferroxidase activity. Its destruction is followed by the release of copper ions that further provoke the formation of $OH^{\cdot}$ and amplify the production of ROS. Nevertheless, it is suggested that leukocytes may actually use this reaction in order to enhance the production of $OH^{\cdot}$ in the acidic environment at the focus of inflammation as a part of the antimicrobial reactions (6, 31, 37). Interestingly, antineutrophil cytoplasmic autoantibody (ANCA) directed to MPO may impede Cp from binding to and inhibiting MPO, thereby promoting a prooxidant state (31). Conversely, lactoferrin is a multifunctional protein, actively secreted by PMN leukocytes. It is an important protector of Cp, capable of binding free Cu ions and thus limiting Cp fragmentation by hydrogen peroxide. This effect depends on the pH level and is being most efficient at 7.4. Overall, Cp interactions regulate local ROS generation during acute inflammation with concomitant protection of adjacent cells from collateral damage (10, 37).

An interesting finding is that Cp-GPI at the surface of human peripheral blood leukocytes (PBL) does not correlate with serum Cp levels, but correlates with ferritin levels (10, 14, 23). Recent studies show that ferroxidase activity is needed in cells expressing Cp-GPI to stabilize ferroportin molecules at the cell surface. Ferroportin becomes degraded in the absence of Cp or when there are sufficient levels of peptide hepcidin (5, 38). Although Cp stabilizes ferroportin and has an antagonistic effect on hepcidin, its raised levels in inflammation are not correlated with iron levels, but the opposite. During inflammation, iron needs to be sequestrated away from pathogens and as a substrate for oxidative stress. Thus, it seems that the action of Cp is reserved for situations of relative hypoxia and iron deficit. In addition, the same as to Cp, the expression of hepcidin is increased by inflammatory cytokines, especially IL-6. Hepcidin controls surface expression of ferroportin 1, by inducing its downregulation and intracellular degradation. Hypoxia, on the other hand, represses hepcidin synthesis (unlike Cp) through the HIF-2α factor (10, 16).

Another important role of Cp is in the tuning of neutrophil apoptosis, which is an essential step in the resolution of acute inflammation. Intact copper-containing Cp and partially proteolyzed forms inhibit delayed spontaneous neutrophil apoptosis, with the intact form showing a pro-survival activity. However, proteolytic forms display a potent TNF-α-induced activation of apoptosis (20).

Ceruloplasmin and copper effects and their regulatory enzymes are engaged during defense against infections caused by bacteria, viruses, and other microorganisms. For example, there are several points where copper metabolism interferes with Influenza A virus (IAV) survival and replication: holo-ceruloplasmin binds to IAV decreasing its infectivity and acting as a trap for virions at early stages, fluctuations in copper concentration reduce IAV reproduction, maintenance of SOD1 function hinders virus replication, etc (4). Apparently, effective replication of many viruses requires an enhanced cellular ROS production, and IAV activity targets the host cell antioxidant defense, among others by significant suppression of SOD1 gene transcription (4).

Interestingly, Cp is significantly increased in exudative pleural effusions compared to other acute phase proteins. Copper levels were significantly higher in both benign and malignant exudates compared to transudate due to fluid overload, and correlated with zinc and manganese levels (39, 40). A study found comparable sensitivity and specificity measures of pleural fluid Cp, and its ratio to the serum Cp, compared to the standardly used Light's

criteria, as well as positive predictive value (98%) of pleural fluid Cp (at ≥ 13.34 mg/dl) and the ratio (at ≥ 0.37) (40).

Copper and Cp in chronic inflammation

Alterations in copper and Cp metabolism are determined in a number of disorders with chronic inflammation and/or immune dysregulation, such as diabetes mellitus (DM), cardiovascular diseases, inflammatory bowel disease (IBD), systemic autoimmune connective tissue diseases, lymphoproliferative disorders, and other (4, 20, 30, 31, 41-44).

Diabetes mellitus and obesity

It is well known that chronic low-grade inflammation is implicated in the pathogenesis of DM type 1 and type 2, which is promoted by obesity and associated with insulin resistance and metabolic dysfunction. An activated immune response may be considered a common antecedent as well as a factor associated with the development of complications in type 2 DM (30, 45, 46). Significantly higher levels of Cp were determined in newly diagnosed type 1 and type 2 DM patients compared to controls (40.69 ± 9.9, 45.05 ± 9.0, and 26.95 ± 4.1 mg/dl, respectively). Interestingly, Cp was markedly reduced after 5-year treatment with oral hypoglycemic drugs (25.73 ± 9.94 mg/dl) (46). In another study, Cp level correlated positively with fasting glucose in healthy subjects along with CRP, however it did not predict the risk of DM incidence (45).

Obesity leads to increased adipocyte oxygen consumption that is due to the elevated free fatty acids level and availability. Increased adipocyte lipolysis results in uncoupled mitochondrial respiration and leads to a state of relative cellular hypoxia that triggers greater expression of HIF-1α and downstream pro-inflamamtory signaling with chronic tissue inflammatory response. Besides, HIF-1α is indicated as a key factor in adipose tissue macrophages (ATM) accumulation and functional change by stimulating chemokine and leukotriene-B4 production (30, 47).

Accumulated ATMs in obese adipose tissue are secreting most of the inflammatory cytokines and are dominant in governing metabolic dysfunction and insulin resistance. In healthy subjects, most of the resident ATM display anti-inflammatory M2-polarization with Th2 supporting effector functions. However, following an adipocyte-related rise in chemokine production ATMs undergo proliferation and phenotypic switching to the M1-like polarized pro-inflammatory state, along with increased recruitment of blood monocytes (M1), neutrophils, and Th1 cells (30, 48).

Differently polarized macrophages differently handle iron and express Cp gene. M1 cells exhibit potent anti-microbial properties with a relatively sealed intracellular iron content, but have a high expression of the Cp gene. On the other hand, M2 cells have ability to recycle iron, higher iron internalization, but downregulated Cp expression (49-51). Therefore, elevated Cp synthesis in M1 cells presumably serves for immune functions and anti-oxdidant defense rather than the iron metabolism. Given the HIF-1α response elements in the Cp gene and increased synthesis in M1 cells, elevated Cp and copper levels are following pro-inflammatory conditions preceding DM.

Higher serum Cp levels were determined in patients with arteriosclerosis. Dysregulation of iron handling and its retention in macrophages promotes the formation of foam cells in atherosclerosis. A study revealed decreased synthesis and activity of Cp in LPS-activated macrophages in the presence of iron and Ox-LDL, suggesting disturbed protective effects of Cp (52). Also, in prolonged inflammatory environment Cp may be submitted to degradation and release of Cu ions, which exhibit pro-oxidant features. Lipid metabolism seems very sensitive to copper imbalance. In this setting, enhanced oxidation of LDL particles had been related to the increased Cp levels, creating additional risk for the development of atherosclerosis (5, 18, 46). By utilizing cell-derived superoxide anion, Cp was shown to be a potent catalyst of LDL oxidation in several cell models (53). LDL contains a few classes of Cu(2+) binding sites, some of which are promoting lipid peroxidation during the propagation phase of atherosclerosis. Several proposed mechanisms explain the copper's ability to modify LDL, but the precise initiating reaction is unresolved (54).

Cardiovascular diseases

Ceruloplasmin has been associated with cardiovascular diseases especially myocardial infarction (MI). It was assessed as a risk factor predicting MI and related cardiovascular complications. Ceruloplasmin and SCL increase transiently after MI, which is consistent with the acute phase response to trauma (6, 11, 42, 53, 55).

Higher Cp levels were observed in patients with ST-elevation myocardial infarction (STEMI) within 12h after onset of chest pain compared to healthy controls (40.1 ± 9.7 vs. 31.4 ± 7.6 mg/dl). These levels significantly correlated with CRP values on the hospital admission, inversely correlated with left ventricular ejection fraction (EF), and were even the most significant marker of ensuing acute heart failure. Ceruloplasmin levels return to about normal after four weeks following infarction. Therefore, a rise in plasma Cp along with other inflammation-sensitive proteins seems to provide a short-term prognostic relevance in patients with systolic dysfunction after acute MI (42).

Most studies so far confirmed a direct relationship between higher serum Cp levels and the incidence of coronary heart disease (CHD) (11). There was a continuous increase in the risk of CHD with increasing Cp, in the middle-aged patients with dyslipidemia (34.9±86 vs. 31.7 ± 77 mg/dl, in controls, $p < 0.001$). The risk was doubled in the highest Cp tertile with an odds ratio of 11.3 in patients with low HDL cholesterol. Besides being determined as an independent risk factor for CHD, its levels were associated with high serum oxidants and decreased anti-oxidant status (11, 56).

Moreover, Cp concentration in CHD was found independently associated with increased risk of

death from cardiovascular and all causes (11, 57, 58). Elevations of CRP and/or Cp were found significantly related to subclinical myocardial necrosis (troponin I < 0.03 ng/mL) in nearly 4000 stable cardiology patients undergoing coronarography. Interestingly, the necrosis was not accompanied with the markers of leukocytes activation but was with the reduction in systemic anti-oxidant enzyme activity, suggesting that pathophysiologic process may be partially independent of leukocyte-mediated action on atherosclerotic plaques. This prodromal subclinical necrosis correlated with a higher long-term risk of major adverse cardiovascular events (MACE) (57). Patients with higher Cp (at 22.0 mg/dL) and MPO (at 322 pg/mL) showed the highest risk of future MACE (58).

Therefore, serum Cp value is a strong candidate for a group of so-called other modifiable non-traditional risk factors which could help to predict some CHD events (11). It seems that elevations of Cp and copper are due to both the inflammation and OxS, which underlie the development of CHD, and therefore may be used as surrogate markers of these processes. Nevertheless, in situations of the overwhelming OxS they could become pro-oxidants and add to the cell damage.

Additionally, copper is lost from the heart during myocardial ischemia. Correspondingly, SCL reached the highest level around the 10^{th} day and gradually recovered until the 21^{st} day. The changes were partially related to the upregulation of a copper transport chaperone - COMMD1 (60).

Investigations of chronic cardiac disorders observed somewhat different results. Particularly, Cp and copper were not found as pronounced markers in these disorders. In patients with chronic heart failure (HF) copper concentration was not significantly correlated with the left ventricular ejection or parameters of the diastolic left ventricular function (61, 62). However, there are some opposite findings, several reports found significant change in Cp levels in chronic cardiac patients. In the study of Cabassi et al. (63) patients with chronic stable HF presented significantly higher levels of Cp (2419 ± 523 vs. 2118 ± 478 nmol/L), copper (21.63 ± 6.77 vs. 16.45 ± 4.87 μmol/L), and nitrotyrosine-bound Cp (11.89 ± 9.29 vs. 5.85 ± 2.01 nmol/L) compared to controls. Low serum ferroxidase I activity was also present and appeared to be due to the oxidative changes in Cp and closely related to low antioxidative capacity. Those with advanced HF had the lowest ferroxidase I activity and the greatest mortality in two years. After adjustment, ferroxidase I activity emerged as a mortality predictor (HR: 2.95). Decreased Cp ferroxidase activity was a consequence of oxidative changes which could affect the progression of left ventricular dysfunction and mortality risk (63).

Inflammatory bowel disease

Inflammatory bowel disease (IBD) is a chronic relapsing inflammatory disease of the intestine with two major subtypes, Crohn's disease (CD) and ulcerative colitis (UC). It is considered a multifactorial disease with prominent inflammation involving extensive recruitment of blood neutrophils and, to a lesser extent, monocytes. Epithelial injury in IBD is characterized by the accumulation of activated neutrophils within epithelial crypts, their transepithelial migration, the release of large amounts of MPO and generation of reactive oxygen and nitrogen species (31, 64).

Elevated MPO with an imbalance between ROS production and antioxidative protection results in OxS which is considered an important pathogenic factor in IBD. The unregulated build-up of inflammatory ROS in the intestinal mucosa easily overwhelms antioxidant enzymes and establishes its persistence. Besides modulation of cellular signaling, ROS are epigenetic regulators that can change microRNAs expression (*e.g.* H_2O_2), which is considered one of the key elements in the pathogenesis of CD (31, 64, 65). The content of two antioxidant enzymes that carry Cu and Zn was found decreased in mucosa of IBD patients, with further deterioration following inflammation (66).

Significantly higher SCL was determined in patients with UC than in healthy controls, both in active disease and remission. Copper levels positively correlated with Cp and C3 and C4 complement (41). During the active inflammation, serum Cp and copper levels are expected to be higher in all IBD patients than in remission, after which they go back to normal. However, in some cases copper deficiency is determined. In a study that explored alteration of serum trace elements in IBD patients, copper insufficiency was present in 15.6% of all IBD cases (20.4% of CD and 7.1% of UC) most of them being in remission (65%). CRP values positively correlated with SCL and Cu/Zn ratio, while systemic inflammation increased the Cu/Zn ratio, suggesting a novel parameter for IBD (66).

There are a few described cases of association between Wilson's disease and UC, where both diseases display high SCL (41). Given the decreased content of antioxidant enzymes and possible alterations in copper metabolism, such as the decreased function of Cu-transporters, it is hypothesized that copper overload and extracellular accumulation take place in the intestinal mucosa, leading to the exacerbation of inflammation and excessive OxS. Extracellular Cu deposits were enriched in sulfur and iron, the latter being known for its ROS forming properties (18, 41).

Recent studies underscore the importance of the damaged epithelial barrier and dysregulated innate immune system in IBD pathogenesis. Macrophages have an important role in maintaining intestinal homeostasis by regulating immune responses to commensal flora. Experimental studies showed that macrophages play a protective role in the development of colitis. A protective anti-inflammatory role of macrophage-derived Cp was demonstrated in a mice model of IBD, but not liver-derived plasma Cp. The Cp-/- mice had exacerbated inflammatory response, increased mortality, and a higher degree of protein oxidation, which is likely due to uncontrolled activity of neutrophils MPO. The recruited macrophages were the primary source of colon Cp with an important role in maintaining intestinal homeostasis (15). Accordingly, several

therapeutic approaches have been investigated and gave beneficial results, such as overexpression of Cu/Zn-SOD in transgenic mice, inhibition of MPO, or inhibition of TNF-α (64, 67).

Interestingly, delayed apoptosis in neutrophils and other pro-inflammatory cells is present in IBD (64). We have already mentioned the role of Cp in the regulation of delayed neutrophil apoptosis. However, this process needs further investigations regarding IBD pathogenesis.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease with multifactorial etiopathogenesis. It is well documented that RA patients have increased serum copper and Cp levels compared to healthy controls, and those with active disease have significantly higher levels than in inactive RA. Copper and Cp might be implicated in the pathogenesis of RA (43, 68-71).

In a group of RA patients, treated with NSAIL, significantly higher values of copper and Cp were determined compared to healthy controls, specifically for total copper (153.9 ± 5.0 vs. 104.0 ± 1.8 μg/dl), immunoreactive Cp (39.0 ± 1.5 vs. 31.3 ± 0.6 mg/dl), oxidase activity of Cp (192.6 ± 6.9 vs. 125.7 ± 2.3 U/L), and the copper/immunoreactive Cp relationship (3.8 ± 0.04 vs. 3.3 ± 0.03 μg/mg). There was a significant negative correlation between Cp concentration and its activity compared to serum OxS markers (TBARS), while without differences in non-Cp bound copper (68).

Copper and Cp positively correlated with CRP values, while copper also correlated with erythrocyte sedimentation rate. Increased SCL was accompanied by the decreased zinc and HDL-c levels in these patients, as well as resulting rise in urinary copper excretion (hypercupreuria) in urine (8.00 vs. 3.98 mg/dl). It should be mentioned, that there was no influence of certain RA treatments on SCL, such as methotrexate, NSAIL, glucocorticoids, vitamin D3, or sulphasalazine (43, 69, 70).

In another study, SCL was significantly higher in patients with RA than healthy controls (110.2 ± 27.8 vs. 81.1 ± 32.1 μg/dl) and patients with osteoarthritis (95.4±27.4 μg/dl). The study determined higher copper levels in the synovial fluid of RA patients compared to healthy (56.6 ± 34.8 vs. 28.1 ± 7.1 μg/dl), which correlated to the lower selenium concentrations (72). Also, levels of Cp, MPO, and thrombin were markedly increased in the synovial fluid of RA patients. Moreover, Cp becomes proteolytically degraded during the inflammation. Also, thrombin cleaves Cp at a site of its inhibitory function toward MPO, thereby interfering with its antioxidant activity (36).

Some studies evaluated and found significant alterations in copper content in the hair of RA patients compared to healthy controls, but their results were contradictory (43, 69). Significantly lower copper levels were determined in erythrocytes and peripheral blood mononuclear leucocytes (PMBL) of RA patients compared to controls. Low erythrocyte Cu content is presumed to be due to the lower activity of SOD enzyme, perhaps as a consequence of its increased utilization in coping with the inflammation-induced OxS. Copper in PBMC was markedly lower in RA patients than in controls (74.3 ± 38.2 vs. 104.2 ± 8.5, μg/10^6 cells) and in those with active compared to inactive disease (58.0 ± 43.2 vs. 86.4 ± 33.2), with inverse relation of SLC and PBMC copper levels (69, 70).

Alterations in copper metabolism in RA seem to be caused by the change in its distribution, due to the accumulation in the inflamed areas and decreased bioavailability in other tissues. Overall, it seems that despite hypercupremia there is a copper deficiency in RA patients. Which, according to some authors may predispose to the higher incidence of infections in these patients (43).

Copper and Cp in leukemia and lymphomas

Numerous studies have found dyshomeostasis of trace elements during carcinogenesis. Owing to its effects on many cellular mechanisms, copper metabolism is often altered in tumors. Here, we briefly review current evidence of copper and Cp relevance in neoplasms of the immune system. In general, patients with malignancies have a significant increase in the total SCL and Cp activity compared to healthy individuals. There are some implications that copper might be included into the pathogenesis of certain forms of cancers due to its necessity for cellular growth (44, 73-76).

Copper and zinc are essential for normal lymphocyte proliferation, maturation and immune functions. Effects of copper and Cp on immunopathogenesis of neoplasia encompass a wide range of potential roles that can be viewed as a part of the antioxidant protection, iron homeostasis, regulation of innate immune response, regulation of angiogenesis and reparation (10, 33). Cellular iron depletion is of particular relevance and an effective host defense mechanism against neoplasia, same as the regulation of redox balance (10, 23). As mentioned, local changes in copper concentration influence the activity of NF-κB and HIF-1α, and thus indirectly on the expression of dozen of genes important for the immune response. Overexpression of HIF-1α and resulting angiogenesis are associated with cancerogenesis, while copper deprivation slows down this process (4, 50). Tumor-associated M2 macrophages (TAM) produce growth factors and have greater release of stored iron, by which they support tumorogenesis. In this regard, the VEGF-C – SOD3 axis was identified as a crucial mediator of tumor survival and metastasis. Although SOD3 levels are usually decreased in tumors, an inverse relationship is found in aggressive tumors, where malignant cells actually create and use SOD3 for survival (49, 52).

Significantly increased SCL is frequently determined in patients with acute and chronic leukemia compared to healthy individuals (73). In a study with a Greek cohort of patients with leukemia, increased copper concentration was the most abundant in cases. Also, the levels were markedly higher in acute leukemia (AML and ALL) compared to chronic (CML, CLL, and lymphoma) (74). In addition, elevated SCL was associated with disease relapse or progression, whereas normal SCL were

found in remission (18 vs. 10 mg/dL) (75). These findings indicate that copper alterations are dependent on tumor activity and may change in response to therapy.

Recent studies performed a high-precision isotopic analysis for determining alterations in trace elements. Blood levels of a heavier copper isotope 65Cu tend to decrease in patients with hematologic malignancies along with the serum enrichment with the light 63Cu isotope (76). In a study of untreated AML patients, although SCL were higher than in controls they were not significantly different (19.5 vs. 16.4 μmol/L). However, the isotopic abundance ratio of copper, 65Cu/63Cu, showed significantly lower levels in patients vs. controls (-0.35 vs. -0.11 per-mille). This is proposed to result from the extensive oxidative chelation of copper heavy isotope by cytosolic lactate in cancer cells (77, 78). Increased intratumoral copper concentrations might promote tumor growth or confer resistance to treatment. For example, the acquisition of resistance to platinum-based treatment was associated with the changes in copper efflux. The copper isotopic ratio is proposed for an early diagnostic use as a cancer biomarker and a follow-up marker (7).

Similar observations were reported in lymphoma patients. Serum copper levels had a very high specificity (95.3%), positive predictive value (92.9%), and a good sensitivity (61.9%) in the diagnosis of Hodgkin lymphoma (HL), with the cut off value at 25.04 μmol/L. However, there was no correlation with the type of HL nor a degree of spreading, assessed through the clinical stage (9). Earlier studies already have shown that the SCL is markedly higher in patients with malignant lymphoma before treatment compared to patients in complete remission (22.97 ± 1.55 vs. 16.36 ± 1.06 μmol/L). And with no marked difference between those in complete remission compared to controls (15.67 ± 0.98 μmol/L) (79).

Importantly, mean copper levels were not significantly different between non-Hodgkin and HL patients. Besides copper (108.5 μg/dL), nickel, chromium and cadmium were found in significantly higher contents in the blood and scalp hair of the lymphoma patients than in healthy controls. In addition, SCL was higher in nodular sclerosing lymphoma patients, in the hair in diffuse large B-cell lymphoma patients, and average copper levels were markedly higher at stage I (44).

Other disorders with copper and Cp alterations

Many other diseases are associated with altered SCL and/or Cp activity, however detailed discussion about all processes is beyond the scope of this article. The copper-based oxidase function of Cp is essential for neuronal development and antioxidant protection in the brain. Also, membrane bound Cp is expressed and secreted by astrocytes and is involved in inflammatory reactions (10, 15, 80). Considering these effects, alterations in copper regulation facilitate the iron accumulation and OxS, leading to neuroinflammation and neurodegeneration. Elevated SLC and/or Cp are frequently reported in Alzheimer's and Parkinson's diseases (15, 20, 25). Hypercupremia is determined in primary biliary cholangitis and sclerosing cholangitis, where copper accumulation may result in toxicity to the basal ganglia (81).

Estrogen and progesterone have well established enhancing effects on Cp synthesis in the liver. Therefore, the levels of Cp rise during pregnancy (almost double), and in females who take oral contraceptives (10, 22, 32). In placenta, there are soluble, GPI-anchored, and specific 4 amino acids longer Cp forms, the later selectively expressed in syncytiotrophoblasts (10). Roles of Cp in implantation and placental function are supposed to enhance iron transport, regulate vascular tone, and have immunomodulatory functions. Ceruloplasmin is determined to decrease the intensity of respiratory burst of neutrophils in the uterus of pregnant females (82). In pre-eclampsia, serum Cp levels are higher than in healthy controls (62 vs. 47 mg/dL), as well as Cp specific antioxidant activity. This increased Cp expression seems to be triggered by the local anoxic response (83).

Conclusion

Copper and Cp involvement in regulation of immune processes are directed toward regulation of iron metabolism, anti- or pro-oxidant activities, microbicidal and protective functions, apoptosis, tissue repair, etc. An increase in total SCL and Cp levels are frequent in the acute phase response to infection and inflammation. Also, they may reflect and take part in the pathophysiological mechanisms involved in DM and MI. Copper dyshomeostasis has been clearly established in many inflammatory autoimmune diseases, cancers, neurological and obstetric disorders. Besides disease understanding, irregularities in SCL could in some situations be utilized as a prognostic and predictive markers, or might be exploited as a therapeutic strategy in the future.

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PLAVI "MOONLIGHTING" PROTEIN U IMUNSKOM ODGOVORU: ULOGE BAKRA I CERULOPLAZMINA U PATOGENEZI ZAPALJENJA I IMUNSKI POSREDOVANIH BOLESTI

Jelena Milenković[1], Branka Đorđević[2], Dijana Stojanović[1], Olivera Dunjić[1], Vanja Petrovski[3]

[1]Univerzitet u Nišu, Medicinski fakultet, Katedra za patofiziologiju, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Departman za biohemiju, Niš, Srbija
[3]Dom zdravlja u Nišu, Niš, Srbija

Kontakt: Jelena Milenković
Bulevar dr Zorana Đinđića 81, 18000 Niš, Srbija
E-mail: jelena.milenkovic@medfak.ni.ac.rs,
jelenaradovic982@gmail.com

Povećanje nivoa serumskog bakra i/ili njegovog glavnog prenosioca u krvi – ceruloplazmina (Cp) čest je nalaz u nekim bolestima kod ljudi. Jedna od najpoznatijih uloga Cp je regulacija ćelijskog unosa gvožđa u situacijama hipoksije. Međutim, pored toga, bakar i Cp uključeni su u brojne fiziološke procese, kao što su redoks balans, regulacija transkripcijskih faktora, rast neurona, određene imunske funkcije: mikrobicidna aktivnost, citoprotektivna barijera, proliferacija limfocita i drugo. Ceruloplazmin je reaktant akutne faze zapaljenja, usled čega njegova koncentracija raste u situacijama akutnih infekcija ili zapaljenja. Takođe, narušavanje homeostaze bakra jasno je ustanovljeno u mnogim zapaljenskim autoimunskom bolestima, malignitetima, neurološkim i opstetričkim bolestima. Promene u metabolizmu bakra i Cp prisutne su u patogenezi dijabetesa melitusa i kardiovaskularnih bolesti. Pored toga, promene serumskog bakra mogu se iskoristiti kao prognostički i prediktivni biomarkeri. Međutim, interpretacija ovih podataka nije dovoljno u upotrebi u rutinskoj kliničkoj praksi. Iz tog razloga, cilj našeg rada bio je prikazati trenutna saznanja i najnovije dokaze o ulogama bakra i Cp, kao dela imunskog odgovora u etiopatogenezi brojnih bolesti, kao i prikazati koristi interpretacije njihovih promenjenih vrednosti.

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Review article

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INSIGHTS INTO THE METABOLISM AND CLINICAL SIGNIFICANCE OF VITAMIN K IN UREMIA: MORE THAN A SUPPLEMENT?

Stefanos Roumeliotis[1,2], Garyfallia Varouktsi[1,2], Ariti Tsinari[1,2], Andrej Veljković[3], Gordana Lazarević[4,5], Zoran Perišić[4,5], Jovan Hadži-Djokić[6], Gordana Kocić[3], Vassilios Liakopoulos[1,2]

Vascular calcification (VC) is a common manifestation of the enhanced Cardiovascular (CV) disease that is observed in Chronic Kidney Disease (CKD). Although the pathogenesis of VC is very complex, recently it became evident that it is the result of the imbalance between calcification promoters and inhibitors, in favor of the former. Matrix Gla Protein (MGP), a powerful inhibitor of VC depends on vitamin K to be fully activated. Epidemiologic data suggest that vitamin K deficiency is highly prevalent in CKD even at the early stages of the disease and correlates tightly with CV outcomes. In animal models, supplementation of vitamin K was accompanied with regression of VC, through activation of MGP. In this review, we aimed to present the existing data regarding the effect of vitamin K supplementation on VC and CV outcome in uremic patients.

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Key words: *arterial calcification, arterial stiffness, cardiovascular disease, chronic kidney disease, vascular calcification, vitamin K*

[1]Division of Nephrology and Hypertension, 1st Department of Internal Medicine, AHEPA Hospital, Thessaloniki, Greece
[2]Aristotle University of Thessaloniki, School of Medicine, Thessaloniki, Greece
[3]University of Niš, Faculty of Medicine, Department of Biochemistry, Niš, Serbia
[4]University Clinical Center Niš, Division of Cardiology, Niš, Serbia
[5]University of Niš, Faculty of Medicine, Niš, Serbia
[6]Serbian Academy of Sciences and Arts, Belgrade, Serbia

Contact: Stefanos Roumeliotis
1 St. Kyriakidi Street, 54636 Thessaloniki, Greece
E-mail: roumeliotis@auth.gr

Introduction

Cardiovascular disease (CVD) still remains the major cause of mortality and morbidity in the chronic kidney disease (CKD) population, accounting for nearly half of the deaths that occur in end stage kidney disease (ESKD) patients receiving dialysis treatment, according to the large national database of United States Renal Data System USRDS (1). Globally, the prevalence of CKD is about 11-13% of the general population but only 0.1-0.3% ends in dialysis, a percentage that is expected to rise dramatically during the next decade (2). This is due to the fact that more of patients at CKD stages 1-2 will die from CVD than progress to ESKD and require dialysis treatment (3). Traditional, Framingham risk factors can only partially explain the increased risk for CVD that is observed in the CKD population. One of the novel mechanisms that is proposed to underlie the occurrence and progression of CVD in CKD might be the calcification of the arteries and cardiac valves (4). Therefore, vascular calcification (VC) or vascular stiffness (VS) might confer to the deleterious CVD burden that is common in uremic patients.

Arterial calcification and stiffness in CKD and ESKD

VC or VS starts in the third decade of life, increases progressively as we age and is a common finding among the healthy aged. In CKD and ESKD, VC and VS start very early (5), are highly prevalent and progress along with deterioration of kidney function. The clinical importance of VC is that the presence of calcium depositions in any artery of the human body (regardless of size or site), confers a 3-4 fold increased risk for CVD morbidity and mortality (6), whereas it is suggested that "we are as old as our arteries are". VC exists in four distinct types: calcification of the media, of the intima layer, of heart valves and calciphylaxis. In CKD, any of these forms might present alone in any combination with other forms (7). Compared to age/gender-matched

controls from the general population, VC in CKD is up to 45 times more pronounced (8), whereas even at CKD stages 1 or 2, VC is detected in 50-90% of all patients (5). In ESKD, VC is more exacerbated, and is prevalent in 90% of all cases; moreover, each extra year on hemodialysis (HD) increases the risk for developing VC by 15% (9). Although VC has been identified over a century ago, the exact pathophysiological mechanisms underlying its occurrence and progression were not fully elucidated until recently.

For more than a hundred years, VC was recognized as a passive, degenerative disorder with no treatment options. This perception was overturned three decades ago, when it became evident that VC was actually an active molecular process, where the first crucial step was the transformation of vascular smooth muscle cells to osteoblastic phenotype (10). Moreover, it was found that this process was modulated by a plethora of molecules and proteins that were implicated in the process of bone formation and either enhanced or abrogated the calcification process. Thus, VC might be the result of the imbalance between calcification promoters and inhibitors, in favor of the former.

Matrix Gla Protein: The natural inhibitor of VC in need of vitamin K

Among various inhibitors of arterial calcification, Matrix Gla Protein (MGP) is the first one to be ever discovered and the most powerful of all (11). MGP is a small molecule with a molecular weight of about 12 kDa, containing 84 amino-acids, 3 serine residues and 4-5 glutamate (Glu) residues. MGP is secreted within the vessel wall and the cartilage and inhibits VC via several pathways (10): firstly, MGP exhibits high affinity to free, circulating calcium, secondly, it directly binds to calcium, phosphorus and hydroxyapatite crystals accumulated in the vessel wall and forms inactive complexes, thirdly, it stimulates the clearance and removal of these complexes from the vessels by macrophages and finally, MGP abrogates the activation of a powerful promoter of VC, bone morphogenetic protein-2, by antagonizing the binding of this molecule to its specific receptors (10, 12, 13). Moreover, there is a synergy between MGP and vitamin D regarding the metabolism of free calcium; MGP keeps the free calcium away from the vessels and vitamin D transfers it to the bones (14). Luo et al., were the first to identify the crucial clinical role of MGP (15); genetically modified rodents that could not express MGP (knockout MGP-/-), were born with natural phenotype but all died within 240 days from their birth due to excessive bleeding caused by rupture of their calcified aorta. Therefore, it became evident that without expressing MGP, life could not be sustained. Additional evidence came from genetic studies showing that mutations in the MGP gene - leading to under expression of MGP - are responsible for the Keutel syndrome, a rare condition where accelerated spontaneous calcification of cartilage, vessels and soft tissues presents (16). Following these, several genetic studies reported a tight association between single nucleotide polymorphisms of the MGP gene and VC/VS or CVD outcomes in various populations, including CKD (17-19). MGP is part of the family of vitamin-k-dependent proteins (VKDPs), which are implicated in coagulation, bone metabolism and cardiovascular health. The common characteristic of all VKDPs is the presence of Glu residues that need to undergo carboxylation, a process that need vitamin K as a cofactor. Only when the inactive Glu residues are fully carboxylated to γ-carboxyglutamate (Gla), VKDPs become biologically active. In addition, MGP has to undergo to another activation process that requires vitamin K as a cofactor, phosphorylation of its serine residues. Therefore, the initial, fully inactive MGP molecule is uncarboxylated and dephosphorylated (dp-ucMGP), which undergoes firstly carboxylation and then phosphorylated to become the fully active form. Only after undergoing these two processes, MGP possesses the ability to act as an inhibitor of VC and an important regulator of circulating calcium (20). However, dp-ucMGP cannot bind to bone morphogenetic protein-2 receptors and no longer has the ability to sweep calcium and hydroxyapatite crystals from the vessel wall (21, 22).

Warfarin, a well-known anticoagulation agent, is a vitamin K antagonist. Since MGP needs vitamin K to become active, the research team from Maastricht found that after 180 days of warfarin supplementation, rats exhibited enhanced VC, due to the impaired carboxylation of MGP (23). After the end of this period, rats were divided to receive warfarin or vitamin K in low or high concentrations for another 240 days and the authors found a 37% regression of VC in the high vitamin K group. This was the first study to show that vitamin K supplementation might prevent or even regress VC, through activation of MGP. During the past decade, dp-ucMGP has been coherently shown to be associated with subclinical, surrogate markers of VC/VS and cardiovascular outcomes in the general population (24-26), in patients with heart failure (27-29) and CVD (30, 31). Since uremia is a state of enhanced arterial calcification, several investigators aimed to explore the potential clinical importance of dp-ucMGP in CKD and ESKD. In a small cohort of pre-dialysis CKD patients, Schurgers et al., were the first to show that dp-ucMGP was correlated with the degree of VC and predicted all-cause mortality (21). In the same study, dp-ucMGP increased progressively across stages of CKD. Similarly, Roumeliotis et al., showed that in a cohort of diabetic CKD patients, dp-ucMGP predicted all-cause/CVD mortality and deterioration of kidney function, after 7 years of follow-up (32). Likewise, other small, cross-sectional studies reported a close association between dp-ucMGP and VC or VS in pre-dialysis CKD cohorts (33, 34). In ESKD, the tight association of dp-ucMGP with CVD outcomes has been repeatedly reported by several studies (35, 36). The major limitations of the forementioned studies include the small sample size, the fact that vitamin K was not directly assessed and the observational design. However, it was coherently shown that dp-ucMGP, reflecting vitamin K deficiency, was a reliable marker of VC in uremic patients.

Vitamin K-the key vitamin for cardiovascular health in uremia

Vitamin K is a family of fat-soluble vitamins playing a pivotal role in coagulation, bone and calcium metabolism. Vitamin K includes two vitamers, K1 or phylloquinone and K2 or menaquinone; in turn, K2 exists in several subtypes, which differ in isoprenyl units and length of side chain. Although the subtype with the longest side chain and the best bioavailability in humans is menaquinone-7 (MK-7) (37), all K forms have the ability to act as cofactors for MGP carboxylation.

Data from large population-based studies - such as the ERGO (38), NHANES II (39), EPIC (40), PREVENT (41) and Danish Diet, Cancer and Health Study (42) - coherently showed that insufficient, dietary vitamin K intake was independently associated with all-cause mortality and CVD in the general population. CKD is a state of subclinical and underdiagnosed vitamin K deficiency which is highly prevalent even at stages 1-2 and gradually increases, as disease progresses to ESKD (43). This could be attributed to several reasons; first, CKD patients often have strict dietary recommendations to avoid green leafy vegetables (that are rich in K1 and potassium) and dietary products (that are rich in K2 and phosphorus) (44), second, the uremic environment might affect the recycling and bioavailability of vitamin K (45), third, certain drug agents that these patients receive (such as phosphate binders and proton pump inhibitors) might further reduce the availability of vitamin K (46) and finally, in uremia, the uptake and metabolism of vitamin K by lipoproteins is significantly impaired (47). In pre-dialysis CKD, in ESKD patients and in kidney transplant recipients, vitamin K deficiency is very common and has been repeatedly associated with the occurrence of CVD outcomes (21, 32, 48, 49). With this background in mind, it was attractive to hypothesize that in uremia, vitamin K supplementation might protect from VC through activation of MGP. To determine the dose of vitamin K that should be supplemented in ESKD patients, Caluwe et al. (50) and Westenfeld et al. (51) conducted two dose-finding studies in HD cohorts. Caluwe et al., divided 200 HD patients to either 360 or 720 or 1080 μg of MK-7 thrice weekly for 60 days and found that plasma dp-ucMGP was decreased by 17%, 33% and 46% in groups, respectively (50). In a study with quite similar design, Westenfeld et al., divided 53 HD patients to either 45 or 135 or 360 μg of MK-7 every day for 45 days and found that plasma dp-ucMGP was reduced by 17.9%, 36.7% and 61.1% in groups, respectively (51). These two studies showed a dose-dependent reduction of dp-ucMGP with increased dose of MK-7 and provided the rationale for interventional, clinical studies in this population. Moreover, it was shown that even a daily dose of 464 μg could not fully restore vitamin K deficiency in HD patients.

Interventional clinical trials of vitamin K supplementation in uremia

To date, seven interventional trials on patients with kidney function impairment have been completed and reported results; two in kidney transplant recipients, two in pre-dialysis CKD and three in dialysis patients. The first study was conducted by Kurnatowksa et al., in 2015 (52), in a cohort of 42 CKD patients at stages 3-5 not receiving dialysis. Patients were randomly allocated to daily, oral supplementation of either vitamin D 10 μg or combination of D with K (MK-7, 90 μg) for 270 days. After the treatment period, the authors reported that compared to the D group, patients in the D+K group exhibited a significant reduction of carotid intima media thickness (cIMT) progression, whereas a non-significant trend towards improvement of coronary artery calcification score in this group was observed. Mansour et al., conducted the KING study, the first interventional trial in kidney transplant recipients (53). In this single arm study, sixty patients received *per os* 360 μg/day of MK-7 for 60 days and at the end of the treatment period the authors found a significant, 14.2% decrease in carotid-femoral pulse wave velocity (PWV). Although these two trials were positive, the ones that followed have reported negative results to date. Oikonomaki et al., conducted a single arm study where they supplemented 52 HD patients with 200 μg/day of MK-7 for 360 days and reported no effect on the Agatston score (54). Likewise, the K4KIDNEYS was a randomized, placebo-controlled trial in a cohort of 159 pre-dialysis CKD patients showing that 400 μg/day of MK-7 for 360 days failed to show any beneficial effect on PWV or abdominal aortic calcification score (55). Similarly, the VALKYRIE study that included 132 HD patients with atrial fibrillation randomly divided to either warfarin, or rivaroxaban or rivaroxaban with vitamin K (200 μg of MK-7, thrice weekly) for 540 days, failed to show any effect of vitamin K on PWV, coronary artery calcification score or calcification of the heart valves (56). In agreement with these results, the RENAKVIT randomized, placebo-controlled trial showed that daily intake of 360 μg MK-7 for 720 days had no effect on VC or VS, in a mixed dialysis cohort of 21 patients either on HD or peritoneal dialysis treatment (57). The most recent randomized, placebo controlled trial that was published is the VIKTORIES trial (58). In this trial, ninety kidney transplant recipients were randomized to either 5 mg of menadiol diphosphate thrice weekly or placebo for 360 days but after the treatment period there was no effect of vitamin K intake on arterial stiffness assessed by MRI-based aortic distensibility or arterial calcification assessed by coronary artery calcium score.

Although the existing studies are very limited, we expect with great interest the results of several, currently ongoing interventional studies in CKD populations, including the Treatment to Reduce Vascular Calcification in Hemodialysis Patients Using Vitamin K (TReVasc-HDK) (59), the Inhibit Progression of Coronary Artery Calcification with Vitamin K1 in Hemodialysis participants (iPACK-HD) (60), the Vitamin K to Slow Progression of Cardio-

vascular Disease Risk in Hemodialysis Patients (Vita-K 'n' CKD) Study (registered in clinicaltrials.gov, with reference number NCT03311321), the Vitamin K1 to Slow Progression of Vascular Calcification in HD Patients (VitaVasK) (61), the Universidad Católica de Salta-Vitamin K2 Supplementation and Vascular Calcification (UCASAL-VITK), which studies for the first time intravenous supplementation of MK-7 in HD patients (registered in clinicaltrials.gov, with reference number NCT04539418) and the Vitamin K in Peritoneal Dialysis (VIKIPEDIA) study, which will be the first to assess MK-7 supplementation in PD (registered in clinicaltrials.gov, with reference number NCT04900610).

Questions that remain, areas of debate and future directions

To date, the existing data do not support that vitamin K supplementation has beneficial effect on VC and CVD in uremic patients. However, the data are limited and derived from studies with major limitations (62). First, although the dose-finding studies by Westenfeld et al. and Caluwe et al., showed that even 464 μg/day of MK-7 failed to restore vitamin K status in HD patients, all aforementioned studies used lower dosages. Secondly, in certain studies the sample size was very small (in RENAKVIT study, only 21 patients completed the study) and thirdly, in other studies (such as the VALKYRIE study), the population included was very old where arteries are in a calcified, mummified status and probably unresponsive to treatment. Finally, in the K4KIDNEYS study the population was probably not vitamin K deficient and the treatment period was rather short.

There are still several questions that remain unanswered: should we treat CKD patients with vitamin K? With which vitamer? Which subform? Which dosage? For how long? And how to monitor the possible beneficial effect? These questions will be hopefully answered when the ongoing, interventional studies report their results.

Conclusion

VC is a common manifestation of CVD in uremia. Although its pathogenesis is very complex, MGP, a powerful inhibitor of VC depends on vitamin K to be fully activated. Epidemiologic data suggest that vitamin K deficiency is highly prevalent in CKD even at the early stages of the disease and correlates tightly with CVD outcomes. In animal models, supplementation of vitamin K (MK-7) was accompanied with regression of VC, through activation of MGP. Until to-day, the data regarding the effect of vitamin K on uremic patients remain limited. Future, larger, interventional randomized placebo-controlled trials are needed in order to draw definite conclusions.

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Pregledni rad

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UVIDI U METABOLIZAM I KLINIČKI ZNAČAJ VITAMINA K KOD UREMIJE: VIŠE OD SUPLEMENTA?

Stefanos Roumeliotis[1,2], Garyfallia Varouktsi[1,2], Ariti Tsinari[1,2], Andrej Veljković[3], Gordana Lazarević[4,5], Zoran Perišić[4,5], Jovan Hadži-Đokić[6], Gordana Kocić[3], Vassilios Liakopoulos[1,2]

[1]Odeljenje za nefrologiju i hipertenziju, Prvi departman interne medicine, AHEPA bolnica, Solun, Grčka
[2]Aristotelov univerzitet u Solunu, Medicinski fakultet, Solun, Grčka
[3]Univerzitet u Nišu, Medicinski fakultet, Katedra za biohemiju, Niš, Srbija
[4]Univerzitetski klinički centar Niš, Klinika za kardiologiju, Niš, Srbija
[5]Univerzitet u Nišu, Medicinski fakultet, Katedra Interna medicina i zdravstvena nega, Niš, Srbija
[6]Srpska akademija nauka i umetnosti, Beograd, Srbija

Kontakt: Stefanos Roumeliotis
Kyriakidi 1, 54636 Solun, Grčka
E-mail: roumeliotis@auth.gr

Vaskularna kalcifikacija (VK) je česta manifestacija pojačane kardiovaskularne bolesti, koja je primećena kod hronične bolesti bubrega (HBB). Iako je patogeneza kradiovaskularnih bolesti kompleksna, u poslednje vreme postalo je evidentno da je posledica disbalansa između promotera kalcifikacije i njenih inhibitora, u korist promotera kalcifikacije. Matriks Gla protein (MGP), moćan inhibitor vaskularne kalcifikacije, zavisi od vitamina K, kako bi bio aktiviran u potpunosti. Epidemiološki podaci sugerišu na to da je prevalencija deficita vitamina K visoka kod hronične bolesti bubrega, čak i u ranim fazama bolesti i u korelaciji je sa kardio-vaskularnim bolestima, kao posledicom. Kod životinja, suplementacijom vitanom K postignuta je regresija kardiovaskularne bolesti, pomoću aktivacije MGP. U ovom pregledu, cilj je da predstavimo postojeće podatke o efektima suplementacija vitamina K na vaskularnu kalcifikaciju i kardiovaskularne bolesti, kod bolesnika sa uremijom.

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Ključne reči: *arterijska kalcifikacija, ukočenost arterija, kardiovaskularne bolesti, hronična bolest bubrega, vaskularna kalcifikacija, vitamin K*

Case report

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A CASE REPORT ON TRAUMATIC DELAYED EPIDURAL HEMATOMA WITH ATYPICAL PRESENTATION

Slavko Živković[1], Jovan Ilić[1], Vesna Nikolov[1,2], Vesna Stokanović[3], Radisav Mitić[1], Marija Djordjević[2], Stefan Todorović[4]

Vertex epidural hematoma (EDH) is an uncommon type of EDH. The management of vertex EDH is a challenge for neurosurgeons, as there is no proper consensus on the proper treatment modality. Our patient had delayed clinical deterioration with the development of paraparesis and deep somnolent state. After an immediate head CT was performed, which showed massive delayed EDH at the vertex, the patient underwent an urgent operation. The postoperative course went satisfactorily with the complete withdrawal of all neurological deficits and control head CT scan showed the complete evacuation of the hematoma. Vertex EDH represents an urgent neurosurgical pathology, which should not be diagnostically overlooked, and by need treated urgently in the operating room.

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Key words: *epidural hematoma, superior sagittal sinus, trauma, dural tenting suture, epidural hemostasis*

[1]University Clinical Center of Niš, Clinic of Neurosurgery, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Niš, Serbia
[3]University Clinical Center of Niš, Clinic of Radiology, Niš, Serbia
[4]University Clinical Center of Niš, Clinic of Neurology, Niš, Serbia

Contact: Jovan Ilić
112/12 Vizantijski Blvd., 18000 Niš, Serbia
E-mail: jovanilic94@gmail.com

Introduction

Epidural hematoma (EDH) represents an urgent neurosurgical pathology and accounts for less than 4% of traumatic brain injuries (TBI) (1). Approximately 60% of traumatic EDHs originate from arterial injury. Data on the occurrence and proportion of traumatic EDH, which are of nonarterial origin, differ to a significant extent and have not been sufficiently reported. Some authors indicate that in about 9.7% of cases the source of bleeding were diploic veins or other venous vessels (2). Regarding other potential causes of nonarterial traumatic EDH, the most common is bleeding from emissary veins, venous dural sinuses, dural venous lakes and rarely, bleeding from arachnoid granulations (2, 3).

Vertex EDHs are recognized as a special entity because they can manifest with an unusual clinical presentation, followed by delayed diagnosis, as well as special consideration for neurosurgical treatment (4, 5). Furthermore, it can be clinically manifested by altered consciousness, headache, unilateral or bilateral weakness of lower limbs, acute intracranial hypertension and hydrocephalus due to blockage of arachnoid granulations and the superior sagittal sinus (SSS) (3).

The management of vertex EDH is a challenge for neurosurgeons, as there is no proper consensus on when to operate and when the supportive care is satisfactory. Furthermore, potential injury and bleeding from the SSS increases surgical risks, which requires careful operative planning and strategy (3, 4, 5).

In this case report, we present a patient with traumatic delayed bifrontoparietal EDH, producing mass effect on the underlying brain parenchyma, emphasizing precaution when operating in the area of the SSS.

Case Report

A 35-year-old male was admitted during early morning hours due to injuries he previously sustained, allegedly at a football match, as a result of hitting his forehead and vertex accidentally on a blunt object. The patient was previously examined in the local hospital, where the head CT was performed, on which the presence of the fracture on the frontal parasagittal right and along sagittal suture

was recorded, measuring 10 cm in length, as well as the presence of a small layer of vertex EDH (Figure 1). After being examined by the neurosurgeon at the admission, it was decided that the patient was going to be treated with supportive therapy. The patient allegedly did not have comorbidities, based on the anamnesis and available medical documentation, and there was allegedly no significant hereditary disease in the family history.

He reported nausea immediately after the injury, and did not vomit or lose consciousness. At the admission to the hospital, clinical examination determined that the patient was conscious, oriented to time, space and person, cardiopulmonary stable, without any recorded gross neurological deficit. Swelling at the site of injury was detected, as well as the presence of subgaleal hematoma.

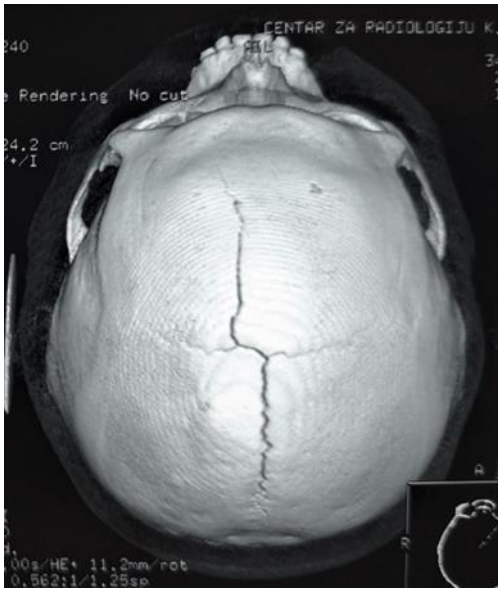

Figure 1. Volume rendering display of calvary with a linear fracture of the frontal bone that continues along the sagittal suture.

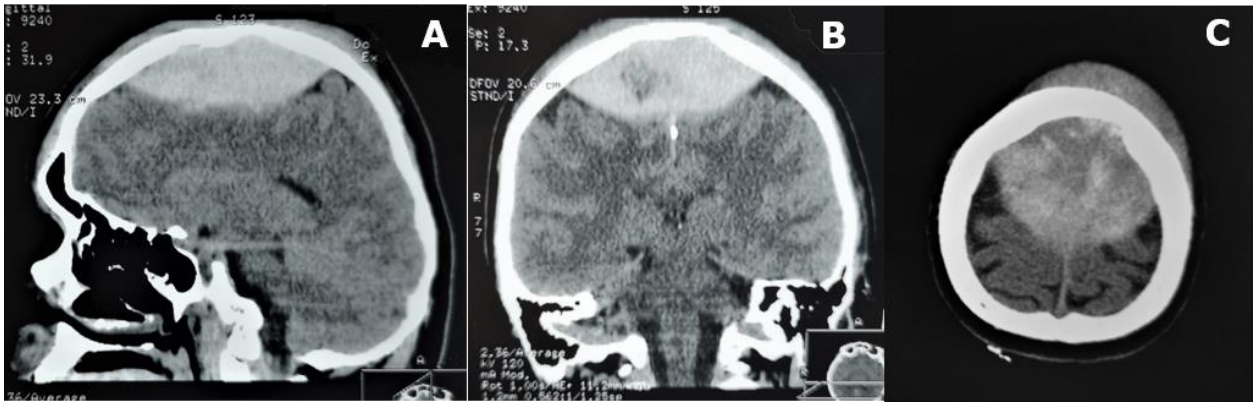

Figure 2. The sagittal (A), coronal (B) and axial (C) CT tomograms show a hyperdense convex collection corresponding to acute bifrontoparietal EDH. The cerebral parenchyma and upper sagittal sinus are displaced caudally.

During the first day of hospitalization, he was treated with analgesics, symptomatic and prophylactic antiepileptic therapy with levetiracetam (Lyvam®) in the dose of 250 mg twice a day. About 20 hours after the admission, the clinical and neurological deterioration of the patient's condition was observed with the development of paraparesis and deep somnolent state. Afterwards, an immediate head CT was performed, which showed that initially seen EDH has enlarged significantly (Figure 2). After urgent preoperative examination, the patient was prepared for surgery.

The patient's head was positioned above the level of the rest of the body and attention was paid to the potential danger of air embolism. In the course of surgery, a linear frontoparietal incision was made sagittally in the length of 12 cm. After careful soft tissue preparation, a frontoparietal fracture was visualized sagittally. The bony lid was removed by 4 burr holes and a large vertex epidural hematoma was encountered. Bleeding from the fracture site was stopped by using a bone wax. Intraoperatively, the bleeding source corresponded to the SSS and the surrounding drainage veins. During the evacuation of the hematoma, the patient bleeded profusely from the torn arachnoid granulations and the SSS, which were tamponaded by using the hemostatic material Surgicel and Liostip. After hemostasis was achieved, an epidural drainage was placed, while the bone lid was fixed back with surgical sutures and returned to the appropriate place.

The postoperative course went satisfactorily with the complete withdrawal of the paraparesis within 6 days, as well as restoration of consciousness, measured by using Glasgow Coma Scale (GCS 15). Control head CT scan was performed after 3 days and showed complete evacuation of the hematoma and minimal gas inclusions in the operating area (Figure 3). At discharge, the patient has reached a full recovery. As for the therapy, he was prescribed with prophylactic anticonvulsant therapy (Levetiracetam-Lyvam® in the dose of 250 mg twice a day), oral analgesic (Metamizole-Analgin® in the dose of 500 mg twice a day) and gastroprotective therapy (Pantoprazole-Nolpaza® in the dose of 40mg once a day). The first check-up by a neurosurgeon was scheduled for 5 days after discharge, when the sutures were removed, and after that in 3 months, but later on every 6 months. During follow-up examinations, the wound healed properly and the patient reached a full recovery.

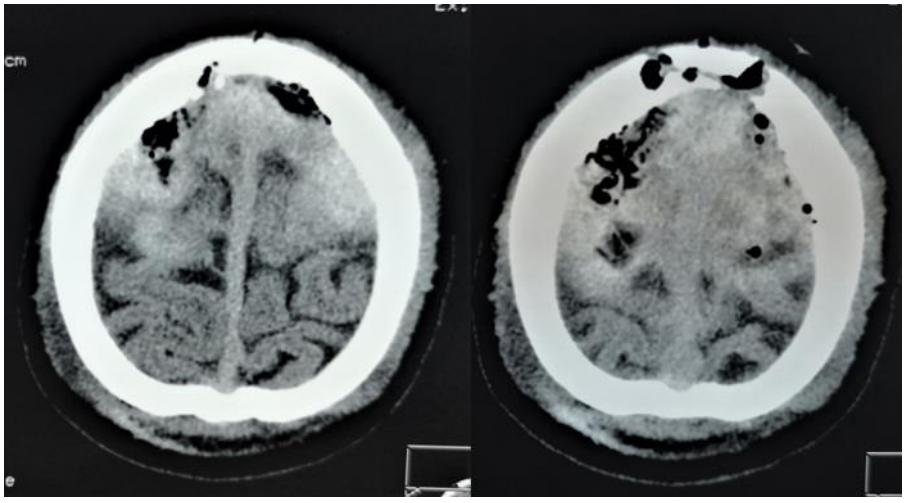

Figure 3. Postoperative axial CT tomograms show complete evacuation of the hematoma and minimal gas inclusions in the operating area.

Discussion

Only a few spontaneous hematomas of this localization have been described in the literature (5). Vertex epidural hematoma is sometimes difficult to detect on head CT with routine sections. The reasons for that might be the small size of hematoma on the initial head CT, the density of the acute hematoma, which is similar to the density of the bone on CT, and scanning plane level may sometimes exclude the vertex (6, 7). A more sensitive method in the detection of epidural hematoma, originating from SSS or dural venous vessels, is MR venography, which accurately detects SSS obstruction in comparison with axial section of CT. Therefore, some authors suggest performing initial CT scans with thinner sections, as well as MR scans (6, 7). In our patient, a layer of epidural hematoma below the SSS was clearly seen on the initial posttraumatic head CT in the sagittal plane.

The delayed deterioration of the clinical condition in our patient could be explained by the potential increase in intracranial pressure (ICP), as well as the mass effect of hematoma on the underlying brain tissue (8). Normal cerebrospinal fluid (CSF) flow involves outflow through arachnoid granulations into dural venous sinuses. When cerebrospinal fluid drainage in the SSS is obstructed, there is a consequent stagnation in the CSF flow, which increases ICP (7). Intense headache, which the patient experienced, could have been caused by stretching of the dura mater around the SSS, which is densely innervated, as well as by the dislocation of the SSS (5). When there is a reasonable suspicion that such condition may develop, attention should be paid to the possible occurrence of visual disturbances and edema of the optic papilla. Moreover; it is possible that patients experience cranial nerve deficits, especially the oculomotor nerve palsy, as well as weakness of the upper extremities and hemiparesis (3, 5), which was not recorded in our case. Paraparesis, which occurred in our patient, may sometimes require differential diagnostic approach for exclusion of potential spinal cord injury (3, 5, 7). Considering that the patient's level of consciousness was reduced and that he had a purely motor paraparesis, which indicates intracranial pathology of the vertex, due to the urgent need for surgery, no additional diagnosis of possible spinal cord injury was made.

The decision about suitable treatment modality should be based upon the characteristics of hematoma, clinical presentation, coronal plain CT scan and neurosurgical assessment. Some authors consider that small hematomas, which do not cause neurological deficits in patients, should be treated conservatively (3, 5, 7). The team of neurosurgeons from our hospital came to the same conclusion, based on the initial clinical presentation of the patient. However, the delayed deterioration of the patient's clinical condition required an urgent surgery.

Special attention in vertex EDH surgery should be redirected to proper hemostasis of the SSS. Bleeding from the SSS can be stopped by applying hemostatic material, reconstruction or ligation. Surgeons avoid ligation of the middle third of the SSS, because the outcome is usually fatal, due to the development of venous obstruction and ICP (7, 9). Surgery in the area of the SSS can trigger the development of SSS thrombosis, a potential life-threatening condition (10).

Dural tenting (DT) around SSS is often used in practice, in order to limit the space for the growth of an epidural hematoma in the event of rebleeding. Moreover, there are disagreements about the benefits of DT and the justification for its use (11, 12). According to some studies, the frequency of epidural rebleeding after DT is between 0.2% and 2.6% for EDH with thickness greater than 3 mm. Furthermore, the risk of formation of subdural hygroma and rebleeding is increased after DT. The enlargement of the subdural space can later lead to stretching and bleeding from the dural bridging veins (11). Proper DT can be a challenge, even for experienced neurosurgeons, with emphasis on visualization of the underlying cortex, in order to avoid dural bridging veins injury. Therefore, according to some authors, it is recommended to use an operating microscope when performing DT (11). In our case, after adequate hemostasis of bleeding from the calvaria, SSS and bridging veins, we decided not to place DT, guided by the above mentioned recommendations and our clinical experience. Postoperative head CT showed that the most of the hematoma was evacuated, and that the small rest was in resorption.

Conclusion

Vertex EDH originating from SSS represent an urgent neurosurgical pathology, which should not be diagnostically overlooked, and if needed, should be treated urgently in the operating room. During the operation, the neurosurgeon must be prepared to prevent potential massive bleeding and the development of air embolism, and to spare the dural bridging veins from Rolandic outflow area.

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PRIKAZ SLUČAJA TRAUMATSKOG ODLOŽENOG EPIDURALNOG HEMATOMA ATIPIČNE LOKALIZACIJE

Slavko Živković[1], Jovan Ilić[1], Vesna Nikolov[1,2], Vesna Stokanović[3], Radisav Mitić[1], Marija Đorđević[2], Stefan Todorović[4]

[1]Univerzitetski klinički centar Niš, Klinika za neurohirurgiju, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Niš, Srbija
[3]Univerzitetski klinički centar Niš, Klinika za radiologiju, Niš, Srbija
[4]Univerzitetski klinički centar Niš, Klinika za neurologiju, Niš, Srbija

Kontakt: Jovan Ilić
Vizantijski bulevar 112/12, 1800 Niš, Srbija
E-mail: jovanilic94@gmail.com

Epiduralni hematom (EDH) verteksa predstavlja retku i atipičnu lokalizaciju EDH. Lečenje EDH verteksa predstavlja izazov za neurohirurge, pošto ne postoji ustaljeno mišljenje i konsenzus o odgovarajućem modalitetu lečenja. Nakon što je prošlo 20 časova od prijema, stanje bolesnika počelo je klinički da se pogoršava, praćeno razvojem parapareze i dubokim somnolentnim stanjem. Ubrzo je izveden CT endokranijuma, pomoću kojeg je primećen veliki bifrontoparijetalni EDH, te je bolesnik podvrgnut hitnom operativnom lečenju. Postoperativni tok prošao je zadovoljavajuće, sa potpunim povlačenjem svih neuroloških deficita, a pomoću CT endokranijuma evidentirana je potpuna evakuacija hematoma. Verteks EDH predstavlja urgentno neurohirurško stanje, koje se ne sme dijagnostički prevideti, sa pripravnošću za hitnim operativnim lečenjem, ukoliko dođe do progresije hematoma.

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Ključne reči: *epiduralni hematom, gornji sagitalni sinus, trauma, duralna suspenzija, epiduralna hemostaza*

Case report

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SQUAMOUS CELL ESOPHAGEAL CARCINOMA IN A YOUNGER FEMALE PATIENT: A CASE REPORT

Andrija Rančić[1], Dragan Stojanov[2], Daniela Benedeto Stojanov[1], Maja Jovičić Milentijević[3], Gordana Petrović[1]

Esophageal cancer is the sixth leading cause of death from malignant diseases. The two most common types of esophageal cancer are squamous cell carcinoma and esophageal adenocarcinoma. Risk factors for the development of these cancers are: age between 60 and 70 years, achalasia, smoking, alcohol consumption, African American origin and others. The main symptoms of esophageal cancer are difficulty swallowing solid and then liquid food, weight loss, pain when swallowing, cachexia, cough or hoarseness. Diagnosis is performed using radiological contrast passage of the esophagus, proximal endoscopy, computed tomography and endoscopic ultrasound. The prognosis is poor with a five-year survival of 15 to 25%. Early detection and cytostatic and surgical treatment are key to successful treatment of this disease. We present a clinical case of an unusual occurrence of esophageal cancer in a younger patient. Also we present the challenges of the diagnostic procedure and the applied therapy.

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Key words: *esophageal cancer, squamous cell carcinoma, gastroscopy*

[1]University Clinical Center Niš, Clinic of Gastroenterology and Hepatology, Niš, Serbia
[2]University Clinical Center Niš, Clinic of Radiology, Niš, Serbia
[3]University Clinical Center Niš, Institute of Pathology, Niš, Serbia

Contact: Andrija Rančić
44 Vase Pelagića St., 18000 Niš, Serbia
E-mail: andrija.m.rancic@gmail.com

Introduction

Esophageal cancer is the sixth leading cause of death from malignant diseases. Five-year survival is about 15 to 25% and the best survival outcomes are achieved in the early stages of the disease (1). There has been a significant incidence increase up to six times worldwide over the past 10 years (2). The two most common types of esophageal cancer are: squamous cell carcinoma and esophageal adenocarcinoma. The incidence rate of esophageal cancer varies by localization (3). Squamous cell type shows a high prevalence in East Asia, southern and eastern Africa, and southern Europe (3, 4). The incidence rate of squamous cell cancer is very low in North America and other parts of Europe (5). Common risk factors for this type are age between 60 and 70, achalasia, smoking, alcohol consumption, African American origin, diet with a high percentage of starch without fruits and vegetables and many more. Adenocarcinoma is more common in the group of patients between 50 to 60 years old, in Caucasians and in male patients, and the main risk factor for the development of this type is prolonged gastroesophageal reflux (1, 6).

The main symptoms of esophageal cancer are: difficulty swallowing, which occurs in 80-96% of patients, primarily solid and later liquid food, weight loss, pain while swallowing, cachexia, cough or hoarseness. Manifest digestive bleeding is rare. Occurrence of anemic syndrome, tracheoesophageal fistula, development of Horner's syndrome (ptosis, miosis and enophthalmos), enlargement of supraclavicular and axillary lymph glands are characteristic of advanced stages of the disease when hematogenous metastases occur (7, 8).

The strategy and the scope of the diagnostic procedure in case of suspected esophageal cancer should be focused on a treatment plan for each individual patient. Proximal endoscopy with biopsy provides adequate diagnosis of the tumor. Abdominal MSCT and endoscopic ultrasound is a good method for obtaining precise tumor localization, and even for staging and classification of tumors according to TNM categories in 80% of cases. In order to rule out bone metastases, bone scintigraphy is often used, while in the case of advanced tumors of the esophagogastric junction, laparoscopy may be performed to rule out peritoneal carcinosis (8, 9).

Case report

We present the case of a 40-year-old female patient who contacted a gastroenterologist due to occasional difficulty in swallowing of solid food and pain when swallowing that first appeared three months earlier. She vomited food on a couple of occasions without the presence of blood, but denied losing weight. Because of enlarged lymph glands on the right side of the neck that the patient noticed by herself, an echosonographic examination of the thyroid gland and soft tissues of the neck was indicated.

Multiple round lymph nodes were described supraclavicularly up to 1 cm in diameter on the left side, and up to 23 mm on the right of the neck (Figure 1). Gastroenterologist then indicated the proximal endoscopy to be performed, which determined infiltrative-vegetative, locally exulcerated constriction of the esophagus located 18 cm from the front teeth. Biopsies were taken and sent for pathohistological analysis (Figure 2). Immunohistochemical analysis revealed the presence of neoplastic cells expressing CK19 and p53 proteins which then led to the diagnosis of squamous cell esophageal carcinoma (Figures 3, 4).

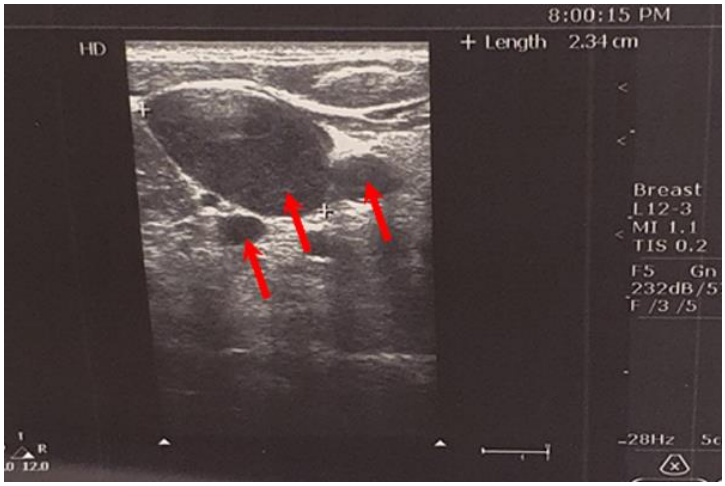

Figure 1. Echosonographic examination of the thyroid gland and soft tissues of the neck - Enlarged lymph glands with a largest diameter of 23 mm on the right side of the neck

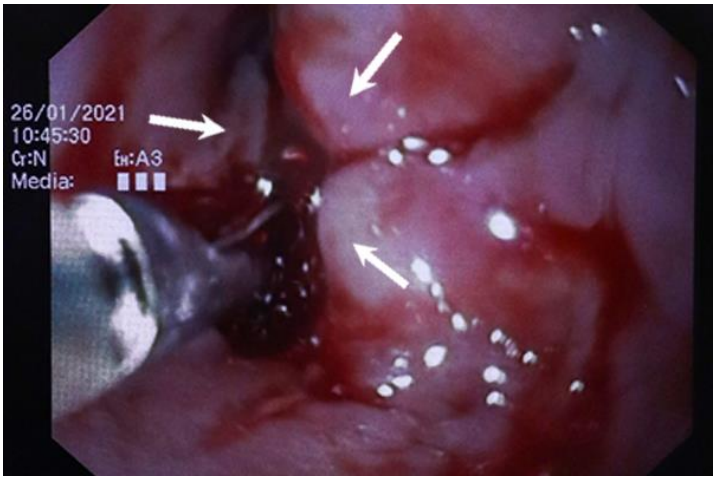

Figure 2. Proximal endoscopy - Infiltrative-vegetative locally exulcerated constriction of the esophagus

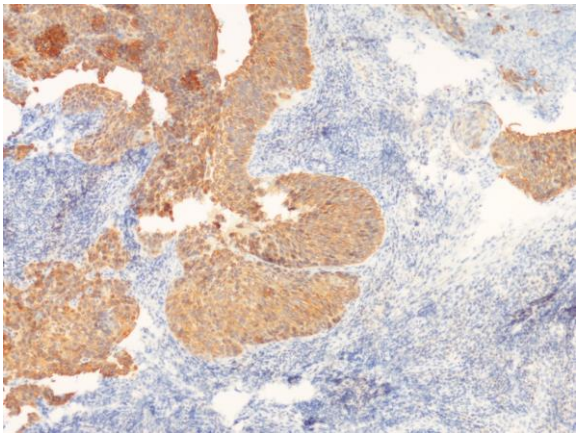

Figure 3. Squamous cell carcinoma – Neoplastic cells express CK19 (immunohistochemistry, 10 x 0.25)

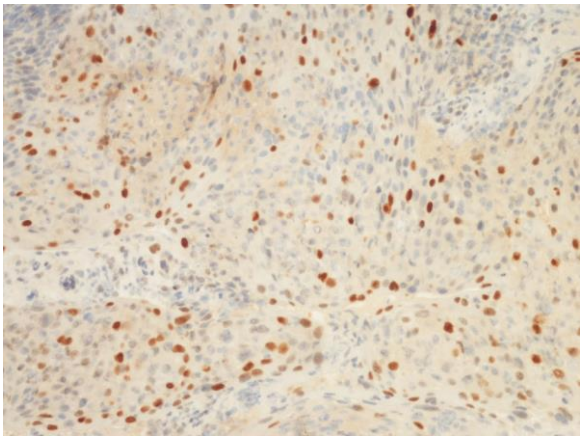

Figure 4. Squamous cell carcinoma – Intranuclear staining of neoplastic cells with p53

The radiography of the esophagus with contrast was performed as a part of preoperative preparation, and computed tomografy of the chest to determine the staging of a cancer. The passage of the esophagus shows a constriction of the lumen in the proximal third of the thoracic segment of the esophagus in a length of 3-4 cm with proximal dilatation of the esophagus (Figure 5). Computed tomography of the chest showed a segmental circumferential thickening of the esophageal wall with necrosis fields at a distance of about 40 mm from the floor of the oral cavity. The described wall thickening has a total AP diameter of up to 30 mm. The described tumefact was localized along the posterior wall of the trachea without the presence of communication (Figures 6, 7). Enlarged lymph nodes up to 27 mm in diameter were observed in the upper mediastinum, and several enlarged, necrotically altered lymph nodes up to 26 mm in size were observed on the right side of the neck. The medical records on the liver and other abdominal organs were normal. The patient was referred to the Clinic of Digestive Surgery of the University Clinical Center of Serbia, where a bronchoscopy was performed and the medical findings were normal. Further neoadjuvant chemoradiotherapy treatment is indicated due to the stage of the disease.

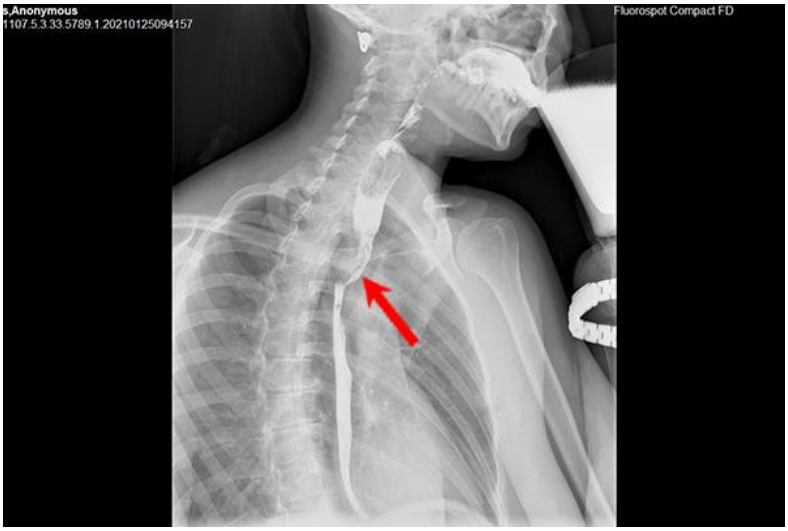

Figure 5. The passage radiography of the esophagus – Constricted lumen of the esophagus with proximal dilation (lateral section)

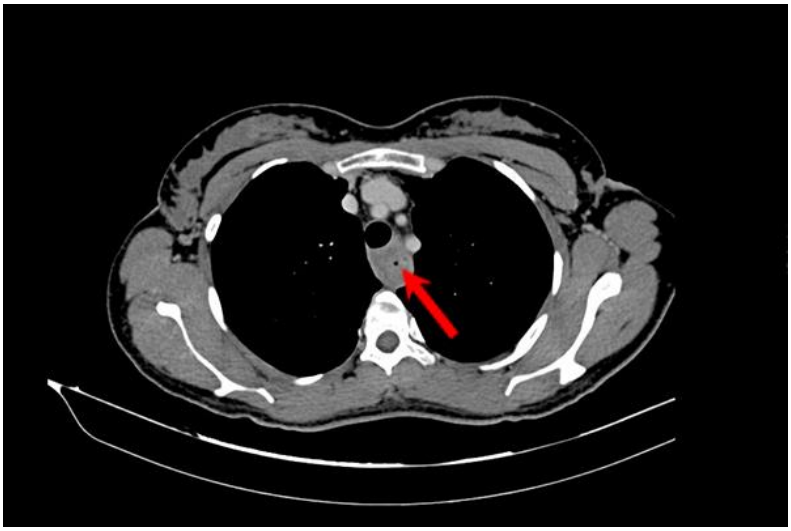

Figure 6. Computed tomography of the chest –
Circumferential constriction of the esophageal lumen

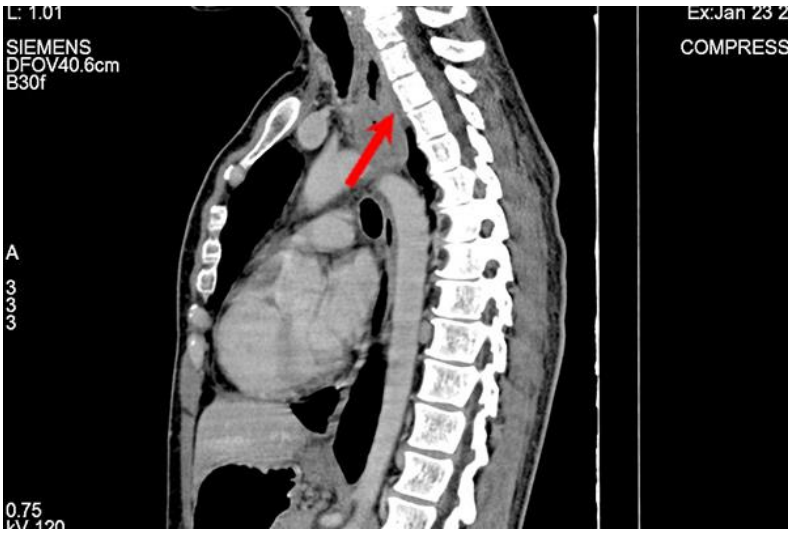

Figure 7. Computed tomography of the chest (lateral section) –
Circumferential constriction of the esophageal lumen

Due to the impossibility of swallowing food, but in order to provide necessary diet of a patient, a surgical nutritional jejunostomy was performed. The intervention went smoothly without complications. The patient was then referred to the gastroenterology council of the University Clinical Center Niš which suggested further implementation of preoperative chemo-radiotherapy.

Discussion

Esophageal cancer ranks eighth most common tumor worldwide. In recent years there has been an increase in the incidence of esophageal cancer in developed countries (10). Esophageal cancer is most commonly diagnosed in age between 60 and 70 years, with a mean age of 67 years. Patients

under the age of 45, as in this case, are only 3.2% of all patients with esophageal cancer (11). Squamous cell carcinoma and esophageal adenocarcinoma together make up more than 95% of all esophageal tumors (10). Major risk factors for squamous cell carcinoma are: older age, smoking, alcohol consumption, nitrosoamine exposure and caustic damage to the esophagus, of which our patient did not have any. Some previous diseases and lesions can be a good basis for the development of this type of cancer, the most significant are Plummer-Vinson syndrome and achalasia, and for adenocarcinoma it is Barrett's esophagus (7). Squamous cell histological type is the most common type of cancer in women, while in men adenocarcinoma is more common. However, the incidence of squamous cell carcinoma is generally higher in men than in women in most countries (12). A study of esophageal cancer in younger individuals done by Dawsey S.P. and coworkers on a sample of 109 patients found a higher incidence of the disease in younger men under 30 years of age compared to women and in a ratio of 1.5 : 1, with the largest number of male patients suffering from squamous cell carcinoma (13). Numerous reports suggest that although younger patients have similar symptoms as older, a much smaller number will seek medical attention, so the diagnosis is made in advanced stages of the disease (14). Saddoughi S. et al. conducted a research on esophageal cancer in a group of patients under 45 and found out that these patients had better overall survival than older patients (15).

Similarly, Wallbohmer et al. found better five-year survival in patients under 50 years of age who were on neoadjuvant therapy (16). However, other publications, such as the one Oezcelik et al., have determined that the younger patients seek for medical help in later stages which was the case with our patient who was diagnosed in the advanced stage of the disease (14). Given the different results of esophageal cancer survival in younger patients, no definitive conclusion can be made about the relationship between age and time of diagnosis. Despite the low five-year survival rate, early diagnosis is crucial in the treatment of esophageal cancer. Survival data are encouraging given that there is an increase among younger people with esophageal cancer (15).

Conclusion

Esophageal cancer is a disease of older age. There is currently an increase in the incidence of the disease among younger people, and the disease most often occurs in men. The squamous cell type is the most common form of this tumor. Five-year survival depends on early diagnosis, age of the patient and applied surgical and oncological therapy. In order to improve the results of treatment, it is necessary to conduct early diagnosis with the appearance of the first symptoms of the disease, with the application of all available diagnostic procedures and the earliest and most aggressive application of therapeutic modalities. It is possible that such an approach would improve the success in combating this vicious disease.

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SKVAMOCELULARNI KARCINOM JEDNJAKA KOD MLAĐE BOLESNICE: PRIKAZ SLUČAJA

Andrija Rančić[1], Dragan Stojanov[2], Daniela Benedeto Stojanov[1], Maja Jovičić Milentijević[3], Gordana Petrović[1]

[1]Univerzitetski klinički centar Niš, Klinika za gastroenterologiju i hepatologiju, Niš, Srbija
[2]Univerzitetski klinički centar Niš, Klinika za radiologiju, Niš, Srbija
[3]Univerzitetski klinički centar Niš, Institut za patologiju, Niš, Srbija

Kontakt: Andrija Rančić
Vase Pelagića 44, 18000 Niš, Srbija
E-mail: andrija.m.rancic@gmail.com

Karcinom jednjaka je šesti vodeći uzrok smrti od malignih bolesti. Dva najčešća tipa karcinoma jednjaka su: skvamocelularni karcinom i adenokarcinom jednjaka. Faktori rizika za nastanak ovih karcinoma su starosni uzrast od 60 do 70 godina, ahalazija, pušenje, konzumiranje alkohola, pripadnost crnoj rasi i dr. Glavni simptomi karcinoma jednjaka su otežano gutanje čvrste, a potom i tečne hrane, gubitak na telesnoj težini, bolovi prilikom gutanja, kaheksija, kašalj ili promuklost. Dijagnostika se vrši primenom radiološke kontrastne pasaže jednjaka, proksimalne endoskopije, kompjuterizovane tomografije i endoskopskog ultrazvuka. Prognoza je loša sa petogodišnjim preživljavanjem od 15% do 25%. Rano otkrivanje i citostatsko i hirurško lečenje ključni su za uspešnu terapiju ove bolesti. Prikazujemo klinički slučaj neuobičajene pojave ezofagealnog karcinoma kod bolesnice mlađe životne dobi. Prikazani su izazovi dijagnostičkog postupka i primenjena terapija.

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Ključne reči: *karcinom jednjaka, skvamocelularni karcinom, gastroskopija*

Case report

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A CASE REPORT ON GIANT CAROTID – OPHTHALMIC ARTERY ANEURYSM

Bojan Stanojević[1], Jovan Ilić[1], Vesna Nikolov[1,2], Aleksandra Aracki Trenkić[3], Boban Jelenković[1,2], Slavko Živković[1], Marija Djordjević[2], Stefan Todorović[4]

Ophthalmic artery (OA) is a potential origin of aneurysms. We present a patient with a giant OA aneurysm, with a sudden onset of psychomotor agitation at the admission. Digital subtraction angiography showed an aneurysm originating from the ophthalmic segment of the left internal carotid artery of irregular shape, with diameters up to 6.5 x 4.5 mm, while the neck of the aneurysm was 4 mm wide. On computed tomography angiography, the aneurysm was seen as much larger and round, up to 4.7 cm in diameter and with a calcified wall. In the further course of treatment, the microcatheter was placed in the lumen of the aneurysm and the embolization spirals were set within. This resulted in a complete exclusion of the aneurysm from the circulation. Endovascular treatment for the giant OA aneurysm, such as coil embolization technique, might be successful for the complete exclusion from circulation of a giant and partially thrombosed aneurysm.

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Key words: *ophthalmic artery, giant aneurysm, mass effect, embolization*

[1]University Clinical Center of Niš, Department of Neurosurgery, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Niš, Serbia
[3]University Clinical Center of Niš, Department of Radiology, Niš, Serbia
[4]University Clinical Center of Niš, Department of Neurology, Niš, Serbia

Contact: Jovan Ilić
112/12 Byzantine Blvd., 18000 Niš, Serbia
E-mail: jovanilic94@gmail.com

Introduction

The part of the internal carotid artery (ICA), running from the distal dural ring to the posterior communicating artery is a potential origin of aneurysms. The incidence of these aneurysms is estimated to be between 0.5% and 11% of all brain aneurysms. One of the most common symptoms of ophthalmic artery (OA) aneurysms is gradual loss of vision, so the goal of the chosen treatment modality should be both exclusion of the aneurysm and preservation or restoring of the sight (1).

Giant intracranial aneurysms, which have diameter greater than 25 mm, make up to 5% of all brain aneurysms (2). Even when performed by an experienced neurosurgeon, the techniques of microsurgical clipping of aneurysm or vessel anastomosis result in high morbidity and pose a high mortality risk because of their complexity (3). Moreover, progress made in the field of endovascular surgery, like the more frequent application of flow diverters, has decreased the number of patients which need an open brain surgery (4). Although the endovascular surgery of OA aneurysm is less invasive, it has lower rate of total exclusion of the aneurysm, it may also not mitigate the visual problems, and the recurrence is more common compared to the microsurgical treatment (4).

In this case report, we present a patient with a giant OA aneurysm, producing mass effect on the surrounding tissue, which was treated with coil embolization, which later led the patient to reaching a full recovery.

Case report

This 64 years old man was presented with a sudden onset of psychomotor agitation which started at the day of the admission. Before being examined by a neurosurgeon, the patient was already examined by neurologist, who stated that the patient had altered mental status and that it was impossible to perform an adequate neurological examination due to the patient's uncooperativeness. Moreover, the neurologist also noted that the patient had no gross neurological deficits on cranial nerves and pyramidal tract. The patient then underwent a brain computerized tomography scan (CT), which showed frontally, on both sides parasagittal, an oval

heterodense, marginally calcified mass, measuring 18 x 43 x 43 mm in diameter, with central calcifications, perifocal edema, and moderate postcontrast enhancement (Figure 1). Therefore, the patient was examined by a neurosurgeon, neurologically assessed again, when it was determined that Glasgow Coma Score (GCS) was 14 at the time of admission, and digital subraction angiography was performed the following day.

Digital subtraction angiography showed an aneurysm originating from the ophthalmic segment of the left ICA of irregular shape, with maximum diameters up to 6.5 x 4.5 mm, while the neck of the aneurysm was 4 mm wide (Figure 2).

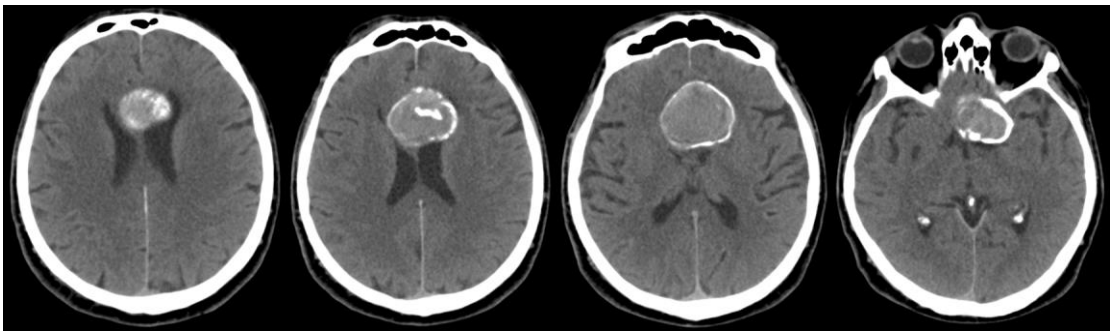

Figure 1. Axial view, brain Computerized tomography (CT) scan study with intravascular contrast agent applied, demonstrating an oval heterodense, marginally calcified mass, measuring 18 x 43 x 43 mm in diameter, with central calcifications, perifocal edema, and moderate postcontrast enhancement

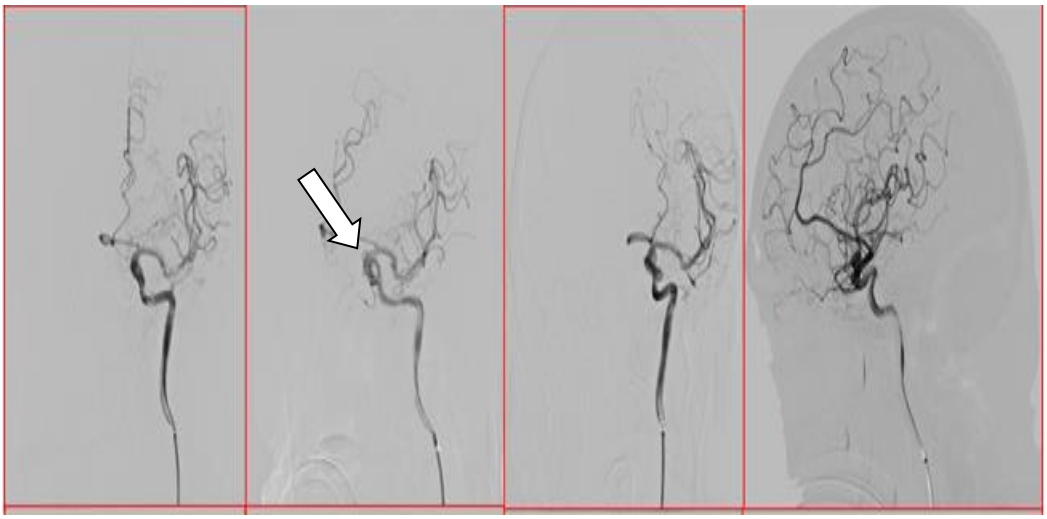

Figure 2. Digital subtraction angiography anteroposterior and lateral view demonstrating an aneurysm originating from the ophthalmic segment of the left ICA before the embolization

In the further course of treatment, the patient was prepared for coiling of the previously detected aneurysm on the ophthalmic segment of the left ICA (Figure 2). The procedure consisted of placing the microcatheter selectively with the tip in the lumen of the aneurysm and setting the embolization spirals within. This resulted in complete exclusion of the aneurysm from the circulation (Figure 3). On the day of the procedure, the patient received 5,000 international units of heparin subcutaneously at 19:00 hours, and from the next day he took regularly acetylsalicylic acid of 75 milligrams dose per day. Patient was admitted to the intensive care unit for overnight observation.

Postoperatively, during the next 4 days of hospitalization, the patient gradually recovered. On the day of discharge, the patient was conscious, oriented with GCS 15 and Glasgow Outcome Score (GOS) was 5, indicating a normal neurological status. Control Magnetic resonance angiography (MRA) was scheduled for 3 months after the day of the procedure.

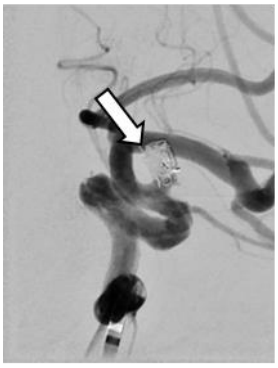

Figure 3. Digital subtraction angiography lateral view demonstrating the complete exclusion from the circulation, with the embolization spirals within the aneurysm

Discussion

It is estimated that giant aneurysms account for about 5% of total number of verified intracranial aneurysms. Although the most of the patients have signs of mass effect lesions due to the diameter of these aneurysms, it is thought that giant intracranial aneurysms have a low risk of rupture (5). In about 50% of cases with giant aneurysms occurs an intra-aneurysmal thrombosis (6). In the present case, the patient probably had an altered mental status due to mass effect of the aneurysm.

If massive mural thrombosis of a sac of giant aneurysm occurs, it does not protect the aneurysm from rupture and bleeding. About one third of the recently ruptured giant intracranial aneurysms had mural thrombosis of a sac (7). Most cases of aneurysms are presented to neurosurgeons after they rupture and cause subarachnoid hemorrhage, but in some cases the size of bigger aneurysms results in mass effect, in which case we should consider neurosurgical procedure where the aneurysm needs to be clipped and the sac starts coagulating, addressing this problem more adequately (8). The patient presented here had no clinical or radiological signs of aneurysm rupture and consequent subarachnoid hemorrhage.

There are a lot of treatment modalities which could be used for patients with giant OA aneurysm, such as clipping, coiling, stenting, external-internal carotid artery bypass with proximal vessel ligation, balloon occlusion (9-12). Surgery of OA aneurysms is challenging even for the most experienced neurosurgeons because these aneurysms are often large and tend to involve the cavernous part of the internal carotid artery (7). It was first planned that the patient would undergo microsurgical clipping due to the favorable dimensions of the neck, and that the thrombosed blood from the aneurysmal sac would be surgically evacuated in order to alleviate the mass effect of on the surrounding structures. Since this intervention would have been very demanding and complicated, and the potential risks to the patient's health would have been high, the other, more suitable treatment was required. Furthermore, more than 50% of the microsurgical vascular operations of the giant aneurysms require occlusion of the proximal, parent artery, accompanied by necessary advanced bypass operation (13).

It has been noted that up to one third of the patients with giant OA aneurysm experience visual deterioration after coil embolization (14). The factors which predict negative outcome in terms of visual deterioration are the size of an aneurysm, previous rupture and visual loss, as well as aneurysm re-treatment (15). Therefore, the appropriate and suitable treatment modality for these patients seems difficult to choose. In our case, we chose endovascular coil embolization, mostly because of the favorable sac–neck ratio, also because it is less invasive and in order to avoid direct optic nerve disturbance. This resulted in the complete exclusion of the aneurysm from the circulation and the patient made a full recovery.

It is considered that if the aneurysm sac has started clotting, with none or mild visual deterioration, then it should be treated with semipermeable stents and coiling, or therapeutic internal carotid artery closure. Guided by the experiences of other eminent neurosurgical centers, and having in mind the current neurological status of the patient, we agreed that the suitable treatment modality for this patient should be endovascular coil embolization. Other possible treatment for this patient could be closing of the ICA with or without a high-flow excimer laser anastomosis, without occlusion, bypass for flow replacement, depending on the balloon test occlusion tolerance (15-17).

As for the follow up of the patient, control MR angiography scheduled for 3 months after the embolization is in accordance with the recommendations and experiences of other neurosurgical centers (15-18).

Conclusion

Endovascular treatment for the giant OA aneurysm, such as coil embolization technique, might be successful for the complete exclusion from circulation of the giant and partially thrombosed aneurysm.

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Prikaz bolesnika

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PRIKAZ SLUČAJA DŽINOVSKE ANEURIZME OFTALMIČKOG SEGMENTA UNUTRAŠNJE KAROTIDNE ARTERIJE

Bojan Stanojević[1], Jovan Ilić[1], Vesna Nikolov[1,2], Aleksandra Aracki Trenkić[3], Boban Jelenković[1,2], Slavko Živković[1], Marija Đorđević[2], Stefan Todorović[4]

[1]Univerzitetski klinički centar Niš, Klinika za neurohirurgiju, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Srbija
[3]Univerzitetski klinički centar Niš, Klinika za radiologiju, Niš, Srbija
[4]Univerzitetski klinički centar Niš, Klinika za neurologiju, Niš, Srbija

Kontakt: Jovan Ilić
Vizantijski bulevar 112/12, 18000 Niš, Srbija
E-mail: jovanilic94@gmail.com

Oftalmička arterija (OA), koja potiče od unutrašnje karotidne arterije, potencijalno je mesto nastanka aneurizmi. U ovom prikazu slučaja opisujemo bolesnika sa džinovskom aneurizmom OA, kod koga se psihomotorna agitacija javila iznenada, na dan prijema na bolničko lečenje. Digitalnom subtrakcionom angiografijom detektovana je aneurizma, koja potiče iz oftalmičkog segmenta leve unutrašnje karotidne arterije, koja je bila nepravilnog oblika, maksimalnog prečnika do 6,5 mm x 4,5 mm, dok je vrat aneurizme bio širok 4 mm. Na prethodnoj CT angiografiji, aneurizma je viđena kao mnogo veća i okruglog oblika, maksimalnog prečnika do 4,7 cm i sa kalcifikovanim zidom. U daljem toku lečenja, postavljen je mikrokateter u lumen aneurizme, a potom su embolizacione spirale postavljene unutar lumena aneurizme. Usledilo je potpuno isključivanje aneurizme iz moždane cirkulacije. Endovaskularno lečenje džinovske aneurizme OA, poput tehnike embolizacije spiralama, može dati potpuno isključenje iz moždane cirkulacije džinovske i delimično trombozirane aneurizme.

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Ključne reči: *oftalmička arterija, džinovska aneurizma, efekat mase, embolizacija*

Review article **UDC: 612.821.7:616.24-008.4:616.831-005.1**
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IS THERE AN ASSOCIATION BETWEEN OBSTRUCTIVE SLEEP APNEA AND CEREBROVASCULAR INSULT?

Zoran Stamenković[1], Tatjana Pejčić[1,2], Dane Krtinić[3,4], Marija Topalović[1], Hristina Jovanović[4], Hristina Trajković[4], Dušanka Kutlešić Kurtović[5]

Obstructive sleep apnea (OSA) is highly prevalent in general population and has a bidirectional association with cerebrovascular insult (CVI), one of the leading causes of global morbidity and mortality. Untreated severe OSA doubles the risk for CVI. OSA may be associated with an increase of all-cause mortality and it may impair neurological outcome in CVI patients. Pathophysiological basis of the association and the possibilities of prevention and improvements of outcomes require further evaluation. Continuous positive airway pressure (CPAP) therapy during sleep is associated with a reduced risk of CVI in OSA patients, but the results are inconsistent. Treatment of post CVI OSA patients with CPAP therapy is recommended as part of the elimination of several risk factors involved in pathogenesis of CVI.
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Key words: *obstructive sleep apnea, cerebrovascular insult, continuous positive airway pressure therapy*

[1]University Clinical Center Niš, Clinic for Lung Diseases, Niš, Serbia
[2]University of Niš, Faculty of Medicine, Department of Internal Medicine, Niš, Serbia
[3]University Clinical Center Niš, Clinic of Oncology, Niš, Serbia
[4]University of Niš, Faculty of Medicine, Department of Pharmacology and Toxicology, Niš, Serbia
[5]University Clinical Center Niš, Clinic of Cardiovascular Diseses, Niš, Serbia

Contact: Zoran Stamenković
48 Dr Zoran Djindjić Blvd., 18000 Niš, Serbia
E-mail: zokinis@live.com

Introduction

Obstructive sleep apnea (OSA) is a potentially fatal condition marked by intermittent hypoxia, sleep fragmentation, and sleep restriction (1). It is a multifactorial disorder and its basic feature is dynamic pharyngeal collapse during sleep (2).

Cerebro-vascular insult (CVI) is defined by the World Health Organization (WHO) as the fast onset of clinical signs of focal or global impairment of brain function, with symptoms lasting 24 hours or more, or with a death outcome, with no other evident cause other than vascular (3).

OSA is highly prevalent in general population and has a bidirectional association with CVI, one of the leading causes of global morbidity and mortality. According to Benjafield et al, 936 million persons aged 30 to 69 years old may have mild to severe OSA (4). CVI is the second leading cause of death and the second most common cause of Disability-Adjusted Life-Years (DALYs) worldwide (5). A typical OSA patient is an obese middle-aged man (6). CVI has a greater impact on younger people, and its prevalence is rising in this age cohort (7).

Pathogenesis

Pathogenic association between OSA and CVI is a direct link between the severity of nocturnal desaturation and intact carotid intima-media and/or atherosclerotic plaques in OSA patients, independent from systemic hypertension finding. During an apnea episode there are significant changes of intracranial pressure and cerebral flow. Mechanical effects of increased intracranial pressure during apnea and hypopnea episode reduce cerebral blood flow for more than 50%, thus predisposing ischemia, being in correlation with the duration of apnea/hypopnea and desaturation degree. Increased plasma fibrinogen and increased platelet aggregation in patients with OSA are associated with increased risk of developing CVI and other cardiovascular consequences. Cardiac arrhythmia, often associated with OSA, is a well-recognized factor of CVI development (6, 8-10).

On the other hand, three non-anatomic contributing factors for OSA onset (arousal threshold, muscle activity, loop gain) are controlled through the

brainstem. So, it is possible that brainstem lesions consequent to CVI may contribute to OSA onset. This can explain why patients with CVI have higher rate of OSA (11). The respiratory centers lesions within the medulla oblongata cause reduced chemosensitivity during wakefulness, sleep, even exercise (12). Several studies have shown that brainstem lesions affect pharyngeal muscle activities, resulting in dysphagia and may contribute to higher rate and severity of OSA. A few studies have investigated how CVI lesion size, regardless of hemorrhagic or ischemic origin, affect the frequency and type of breathing disturbance in sleep. Ahn et al. showed that bilateral hemisphere lesions resulted in significantly higher OSA severity in comparison to CVIs that occurred in a single region (13).

However, pathophysiological basis of the association and the possibilities of prevention and improvements of outcomes require further evaluation.

Current knowledge

Four scientific societies: European Academy of Neurology-EAN, European Respiratory Society-ERS, European Stroke Organization-ESO, European Sleep Research Society-ESRS, have established a working group of 15 experts in the fields of neurology, stroke, respiratory medicine and sleep medicine to critically evaluate evidence of potential links btween sleep and stroke diseases and the importance of therapy. A comprehensive search of the literature published between 1990 and 2019 was conducted. A total of 12.870 studies were reviewed, with 88 of them meeting the rigorous inclusion requirements. There were 13 research questions that were answered. The evidentiary basis for linking OSA to CVI is strongest in general, and it supports active diagnosis and treatment.

When it comes to the impact of OSA on CVI, it was concluded that untreated severe OSA doubles the risk for CVI, which is especially significant in younger and middle-aged patients. The available evidence, although still insufficient, indicates an increased risk of OSA caused by CVI in patients with coronary artery disease or atrial fibrillation, with the possible exception of elderly patients. Although observational cohort studies suggest that continuous positive pressure (CPAP) treatment during sleep is associated with reduced risk of CVI in OSA patients, the results are inconsistent, and meta-analyzes of RCTs do not find this association, however, at the same time, it is pointed out that patients adherent to CPAP therapy (> 4 h daily) may benefit. There is not enough evidence of the effectiveness of treatment options other than CPAP treatment.

At the second part of the association, the impact of CVI on OSA, it is pointed out that the prevalence of OSA in patients with CVI is high, about 50%, and 30% have severe OSA (AHI > 30). Respiratory polygraphy is sufficient to assess the presence and severity of OSA in these patients. The association between CVI parameters (type, severity, topography, etiology) and OSA severity in patients with CVI, as well as the predictors of OSA in patients with CVI, are poorly understood. The evolution of OSA throughout time is unpredictable. OSA is linked to an increased chance of recurrence of CVI or a transient ischemic attack (TIA), as well as an increased risk of death from any cause and a deteriorating neurological prognosis. Current evidence suggests that CPAP therapy is possible in patients with CVI and OSA. CPAP therapy in these patients can improve drowsiness, depression and neurological recovery and eliminate or improve several risk factors such as anticoagulant effect, atrial fibrillation, better control of hypertension and dyslipidemia, weight loss. Acceptance of CPAP therapy in acute CVI is limited, but when CPAP therapy is accepted, the compliance may be satisfactory. There is insufficient evidence to support the impact of other OSA treatment modalities on CVI outcome (14).

Conclusion

OSA increases the risk of CVI development. The outcome of CVI is worsened by OSA. OSA and CVI have a bidirectional relationship, approximately half of individuals with CVI also have OSA. CPAP therapy may help to lower the risk of CVI development and improve the prognosis of the condition. Future studies should look at the impact of different OSA phenotypes on this association, in addition to understanding the pathophysiological basis of the link between OSA and CVI.

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DA LI POSTOJI VEZA IZMEĐU OPSTRUKTIVNE APNEJE U SNU I CEREBROVASKULARNOG INSULTA?

Zoran Stamenković[1], Tatjana Pejčić[1,2], Dane Krtinić[3,4], Marija Topalović[1], Hristina Jovanović[4], Hristina Trajković[4], Dušanka Kutlešić Kurtović[5]

[1]Univerzitetski klinički centar Niš, Klinika za plućne bolesti, Niš, Srbija
[2]Univerzitet u Nišu, Medicinski fakultet, Katedra za internu medicinu, Niš, Srbija
[3]Univerzitetski klinički centar Niš, Klinika za onkologiju, Niš, Srbija
[4]Univerzitet u Nišu, Medicinski fakultet, Katedra za farmakologiju sa toksikologijom, Niš, Srbija
[5]Univerzitetski klinički centar Niš, Klinika za kardiovaskularne bolesti, Niš, Srbija

Kontakt: Zoran Stamenković
Bulevar dr Zorana Đinđića 48, 18000 Niš, Srbija
E-mail: zokinis@live.com

Opstruktivna apneja u snu (obstructive sleep apnea – OSA) veoma je rasprostranjena u opštoj populaciji i ima dvosmernu vezu sa cerebrovaskularnim insultom (CVI), jednim od vodećih uzroka morbiditeta i mortaliteta globalno. Nelečena teška OSA udvostručuje rizik od CVI-a. OSA može biti povezana sa povećanjem mortaliteta proisteklog iz svih uzroka i može negativno uticati na neurološki ishod kod bolesnika sa CVI-om. Patofiziološka osnova udruženosti i mogućnost prevencije i poboljšanja ishoda zahtevaju dalju evaluaciju. Terapija kontinuiranim pozitivnim pritiskom (CPAP) u disajnim putevima tokom sna povezana je sa smanjenim rizikom od CVI-a kod OSA bolesnika, ali rezultati su varijabilni. Lečenje post CVI OSA bolesnika CPAP terapijom preporučuje se u sklopu otklanjanja više faktora rizika uključenih u patogenezu CVI-a.

Acta Medica Medianae 2022;61(2):98-101.

Ključne reči: *opstruktivna apneja u snu, cerebrovaskularni insult, terapija kontinuiranim pozitivnim pritiskom tokom sna*

JEDINSTVENI KRITERIJUMI ZA OBJAVLJIVANJE NAUČNIH RADOVA U BIOMEDICINSKIM ČASOPISIMA

Ideja o postavljanju jedinstvenih kriterijuma za objavljivanje radova u časopisima za biomedicinske nauke iskristalisana je 1978. godine u Vankuveru. Ovi kriterijumi za rukopise, uključujući pravila za pisanje bibliografije, prvi put su objavljeni 1979. godine. Vankuverska grupa je vremenom prerasla u Međunarodni komitet urednika medicinskih časopisa — International Committee of Medical Journal Editors (ICMJE). Trenutno je na snazi peta revizija kriterijuma za objavljivanje radova u biomedicinskim časopisima, doneta 1997. godine.

Kriterijumi za citiranje i navođenje referenci

Reference se obeležavaju arapskim brojevima u zagradama, pri čemu se reference obele-žavaju brojevima onim redosledom kojim se pojavljuju u tekstu. Reference citirane jedino u tabelama ili legen-di moraju se obeležiti brojem u skladu sa redosledom pojavljivanja u tekstu.

Naslove medicinskih časopisa treba pisati u skraćenom obliku onako kako su navedeni u poglavlju **List of Journals Indexed in Index Medicus**. Lista skraćenih naziva medicinskih časopisa objavljuje se svake godine u januarskom broju ***Index Medicusa***. Ova lista se takođe može naći na adresi **www.nlm.nih.gov**

Izbegavati upotrebu apstrakata kao referenci, već koristiti samo izvorne tekstove (*in extenso* članci). Reference koje se odnose na radove koji su prihvaćeni, ali još nisu odštampani, treba označiti sa "u štampi", pri čemu autor mora imati pismeno odobrenje da citira takve radove i da priloži pismeni dokaz da je citirani rad prihvaćen za štampu. Informacije iz rukopisa koji nisu prihvaćeni za štampanje mogu se citirati u tekstu kao "neobjavljeni rezultati", ali sa pismenom dozvolom autora.

Izbegavati citiranje prethodnih saopštenja (personal communication) ukoliko ona ne obezbeđuju esencijalne rezultate koji još nigde nisu objavljeni. U ovom slučaju, neophodno je u zagradi navesti ime osobe i datum usmenog saopštenja rezultata. Za objavljivanje ovih podataka neophodno je pismeno odobrenje autora.

Kriterijumi za pisanje referenci korišćenih u radu

U ovom pregledu biće obrađena pravila za pi-sanje literaturnih referenci samo za najčešće korišćene tipove publikacija.

Članci u časopisima

1. Standardni članak u časopisu

Navesti prvih šest autora, ukoliko ih je više iza šestog dodati **et al.** ukoliko je referenca na engleskom jeziku ili **i sar.** ukoliko je referenca na srpskom jeziku.

Vega KJ, Pina I, Krevsky B. Heart transplantation is associated with an increased risk for pancreatobiliary disease. Ann Intern Med 1996; 124(11):980-3.

Parkin DM, Clayton D, Black RJ, Masuyer E, Friedl HP, Ivanov E, et al. Childhood–leukaemia in Europe after Chernobyl: 5 year follow-up. Br J Cancer 1996;73:1006-12.

2. Organizacija kao autor

The Cardiac Society of Australia and New Zealand. Clinical exercise stress testing. Safety and performance guidelines. Med J Aust 1996;164:282-4.

3. Članak bez poznatih autora

Cancer in South Africa (editorial). S Afr Med J 1994;84:15.

4. Volumen sa suplementom

Shen HM, Zhang QF. Risk assessment of nickel carcinogenicity and occupational lung cancer. Environ Health Perspect 1994; 102 Suppl 1:275-82.

5. Broj sa suplementom

Payne DK, Sullivan MD, Massie MJ. Women's psychological reactions to breast cancer. Semin Oncol 1996;23(1 Suppl 2):89-97.

6. Volumen sa više delova

Ozben T, Nacitarhan S, Tuncer N. Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. Ann Clin Biochem 1995;32(Pt 3):303-6.

7. Broj sa više delova

Poole GH, Mills SM. One hundred consecutive cases of flap lacerations of the leg in ageing patients. N Z Med J 1994;107(986 Pt 1):377-8.

8. Časopisi sa brojem bez volumena

Turan I, Wredmark T, Fellander-Tsai L. Arthroscopic ankle arthrodesis in rheumatoid arthritis. Clin Orthop 1995;(320):110-4.

9. Časopisi bez volumena i broja

Browell DA, Lennard TW. Immunologic status of the cancer patient and the effects of blood transfusion on antitumor responses. Curr Opin Gen Surg 1993:325-33.

10. Reference u obliku apstrakta ili prethodnih saopštenja

Enzensberger W, Fischer PA. Metronome in Parkinson's disease (letter) Lancet 1996;347:1337.

Clement J, De Bock R. Hematological complica–tions of hantavirus nephropathy (HVN) (abstract). Kidney Int 1992; 42:1285.

Udžbenici i monografije

11. Monografija

Ringsven MK, Bond D. Gerontology and leadership skills for nurses. 2nd ed. Albany (NY): Delmar Publishers; 1996.

12. Autori kao urednici

Norman IJ, Redfern SJ, editors. Mental health care for elderly people. New York: Churchill Livingstone; 1996.

13. Organizacija kao autor i izdavač

Institute of Medicine (US). Looking at the future of the Medicaid program. Washington: The Institute; 1992.

14. Poglavlje u knjizi

Phillips SJ, Whisnant JP. Hypertension and stroke. In: Laragh JH, Brenner BM, editors. Hypertension: pathophysiology, diagnosis, and management. 2nd ed. New York: Raven Press; 1995. p. 465-78.

15. Conference proceedings

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15-19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

16. Conference paper

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors.

MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561-5.

17. Istraživački ili tehnički izveštaji

Službeni izveštaji (Issued by funding / sponsoring agency):

Smith P, Golladay K. Payment for durable medical equipment billed during skilled nursing facility stays. Final report. Dallas (TX): Dept. of Health and Human Services (US), Office of Evaluation and Inspections; 1994 Oct. Report No.: HHSIGOEI69200860.

Sponzorisani izveštaji (Issued by performing agency)

Field MJ, Tranquada RE, Feasley JC, editors. Health services research: work force and educational issues. Washington: National Academy Press; 1995. Contract No.: AHCPR282942008. Sponsored by the Agency for Health Care Policy and Research.

18. Magistarske i doktorske disertacije

Kaplan SJ. Post-hospital home health care: the elderly's access and utilization [dissertation]. St. Louis (MO): Washington Univ.; 1995.

Druge vrste publikovanog materijala

Neobjavljeni materijal

19. U štampi (In press)

Leshner AI. Molecular mechanisms of cocaine addiction. N Engl J Med. In press 1996.

Elektronski zapisi

20. Internet članak u elektronskom formatu

Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar "cited 1996 Jun 5"; 1(1)(24 screens). Available from: URL: http://www.cdc.gov/ncidod/EID/eid.htm

21. Monografija u elektronskom formatu

CDI, clinical dermatology illustrated (monograph on CD-ROM). Reeves JRT, Maibach H. CMEA Multimedia Group, producers. 2nd ed. Version 2.0. San Diego: CMEA; 1995.

22. Kompjuterski podaci

Hemodynamics III: the ups and downs of hemodynamics (computer program). Version 2.2. Orlando (FL): Computerized Educational Systems; 1993.

PROPOZICIJE ZA PISANJE RADOVA U ACTA MEDICA MEDIANAE

Acta Medica Medianae (AMM) je tematski časopis iz oblasti medicinskih nauka. Časopis objavljuje originalne radove koji nisu prethodno publikovani.

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Priloge u vidu teksta, tabela i ilustracija (grafikoni, crteži i dr.) ne unositi u tekst članka, već na kraju teksta, na posebnim stranicama obeleženim u gor-njem levom uglu sa "Tabela, Grafikon, Ilustracija" i arapskim brojem redosledom pojavljivanja u tekstu (npr. Tabela 1, Grafikon 1 i dr.) i svakoj se daje kratak naslov. Kratka objašnjenja i skraćenice daju se u fusnoti. Za fusnotu koristiti sledeće simbole: *, **, ***, #, ##, ###, …itd. Tabele, grafikone i ilustracije treba praviti korišćenjem nekog od programa iz Microsoft Office paketa. Izbegavati upotrebu boja kod izrade grafika.

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SECRETARIAT

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