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Review article

Urinary Biomarkers as Early Indicators of Acute Kidney Injury in Neonates with Perinatal Asphyxia

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SUMMARY

Introduction/Aim. Perinatal asphyxia (PA) is a condition in which there is a decreased or interrupted blood and oxygen supply to the tissues of the fetus, i.e., the newborn, immediately before, during, or immediately after delivery. It constitutes a significant cause of mortality, accounting for 23-24% of all neonatal deaths. The estimated global incidence of perinatal hypoxia is approximately 0.5% of the total number of live births at gestational age over 36 weeks. PA negatively impacts the entire organism, especially metabolically demanding tissues. Due to the sensitivity of the kidneys to oxygen deprivation, acute kidney injury (AKI) can develop within the first 24 hours of the ischemic episode. Prolonged ischemia may lead to irreversible cortical necrosis. Early recognition of AKI is crucial for adequate fluid and electrolyte replacement, as the action of pre-renal etiological factors is a dynamic process with a reversible onset. However, AKI represents a poor prognostic sign, with higher mortality in neonates who develop AKI after perinatal asphyxia, and up to 40% of survivors may have permanent kidney damage. Given the specificity of both the population and the clinical entity, there is a clear need for newer, more sensitive, and specific biomarkers of renal function. The aim of the paper was to review the most significant urinary biomarkers in neonates with perinatal asphyxia that could be crucial for early detection of renal impairment.
Methods. Analysis of scientific and professional papers published in the last ten years in international scientific and professional journals available in the PubMed database.
Conclusion. When considering a potential biochemical marker, the type of biological sample in which it is quantified is a crucial characteristic that must be taken into account. For newborns, obtaining a sample non-invasively is of utmost importance. In this context, urine analysis emerges as a good choice. Metabolites in the urine of PA patients have been proven significant for monitoring the renal function. Unfortunately, urine as a biological sample has the drawback that it cannot be obtained immediately after birth, and a significant number of neonates due to pre-existing renal damage may be anuric.

Keywords: **perinatal asphyxia, acute kidney injury, urinary biomarkers**

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INTRODUCTION

Asphyxia is a condition in which pulmonary, or in the case of the fetus, placental gas exchange is significantly compromised or completely interrupted. It is a state induced by hypoxia and/or ischemia, associated with lactic acidosis. Hypoxia or anoxia signifies a partial or complete lack of oxygen in tissues or blood. Ischemia represents a reduction (partial ischemia) or cessation (complete ischemia) of circulation in organs, compromising the delivery of oxygen and substrates to tissues (1). Perinatal asphyxia (PA) is a condition in which there is a decreased or interrupted blood and oxygen supply to the tissues of the fetus, i.e. the newborn, immediately before, during, or immediately after delivery. PA is a significant cause of mortality, accounting for 23-24% of all lethal outcomes in the neonatal period. PA negatively impacts the entire organism, especially metabolically demanding tissues of the central nervous system, heart, kidneys, and gastrointestinal tract. It significantly affects the mortality and morbidity of newborns. The estimated global incidence of perinatal hypoxia is approximately 0.5% of the total number of live births at gestational age over 36 weeks (2).

PA can result from maternal conditions (severe anemia, severe hypoxia, pre-eclampsia and eclampsia, trauma, shock, coagulation disorders), various pathological conditions of the fetus/newborn (cyanogenic heart defects, persistent pulmonary hypertension of the newborn, cardiomyopathy, shock of various etiologies, fetal hydrops, infections, coagulopathies), complications related to the placenta and umbilical cord (placental abruption, placenta previa, umbilical cord knot), but it can also be idiopathic (3).

Hypoxia triggers complex reflex responses aimed at redirecting blood to priority organs—the brain, heart, and adrenal glands, at the expense of other organs. Since the kidneys are very sensitive to oxygen deprivation, acute kidney injury (AKI) can develop within the first 24 hours of the ischemic episode. If ischemia is prolonged, irreversible cortical necrosis may occur. Early recognition of AKI is crucial for adequate fluid and electrolyte replacement, as the action of pre-renal etiological factors is a dynamic process with a reversible onset. However, AKI represents a poor prognostic sign. Mortality is higher in neonates who develop AKI after perinatal asphyxia, and up to 40% of survivors may have permanent kidney damage (1, 4).

Clinical-biochemical criteria for defining renal damage in neonates exposed to PA are inconsistent. According to the study by Shah et al. (5), these are: anuria or oliguria (< 1 mL/kg/h) for 24 hours or longer with serum creatinine values >100 μmol/L; or anuria/oliguria for 36 hours; or any serum creatinine value >125 μmol/L; or multiple serial measurements indicating postnatal increase in creatinine values.

Given the specificity of both the population and the clinical entity, there is a clear need for newer, more sensitive, and specific biomarkers of renal function. When considering a potential biochemical marker, the type of biological sample in which it is quantified is a crucial characteristic that must be taken into account. For newborns, obtaining a sample non-invasively is of utmost importance. In this context, urine analysis emerges as a good choice. Metabolites in the urine of PA patients have been proven significant for monitoring the renal function. Unfortunately, urine as a biological sample has the drawback that it cannot be obtained immediately after birth, and a significant number of neonates due to pre-existing renal damage may be anuric (6).

AIM

The aim of the paper was to review the most significant urinary biomarkers in neonates with perinatal asphyxia that could be crucial for early detection of renal impairment through the analysis of scientific and professional papers published in the last ten years in international scientific and professional journals available in the PubMed database.

Cystatin-C (CysC)

CysC is a low-molecular-weight protein with a mass of 13 kDa produced by nucleated cells, functioning as a protease inhibitor. It is excreted by the kidneys through glomerular filtration, but it is not present in significant quantities in urine because proximal tubular cells almost completely absorb it (7). CysC does not cross the hematoplacental barrier, making it a significant indicator of renal function in newborns in the early postnatal period. It is superior to creatinine, which crosses the hematoplacental barrier and, therefore, cannot detect antenatal kidney damage, as maternal kidneys can clear the fetal creatinine. Since it is almost entirely reabsorbed,

serum CysC values cannot be considered a direct marker of renal damage but rather an indicator of changes in glomerular filtration rate. In contrast, urinary CysC values are a direct indicator of proximal tubular damage (7, 8).

Li et al. (9) identify urinary CysC as significant for detecting renal damage and predicting AKI in neonates with confirmed perinatal asphyxia. Sarafidis et al. (10) demonstrated that serum CysC values were elevated in neonates only on the first day after the asphyctic episode compared to the control group, while urinary CysC values were elevated on the first, third, and tenth day. Khosravi et al. (11) state that in their study of 55 patients diagnosed with AKI in the neonatal intensive care unit, CysC values had predictive significance for the development of AKI with sensitivity of up to 98.2%. Additionally, CysC values correlate with the severity of hypoxic ischemic encephalopathy (HIE). Studies on the utility of CysC in clinical practice conducted on an older population are not relevant in neonatology, as urinary CysC values change over time, decreasing with kidney maturation (12).

Neutrophil gelatinase–associated lipocalin (NGAL)

Neutrophil gelatinase–associated lipocalin (NGAL) is a protein with a mass of 21 kDa that represents lipocalin 2 or siderocalin covalently bound to the gelatinase of neutrophil granulocytes. It is released by specific granules of neutrophil granulocytes after activation. The values of this biomarker are elevated in the serum and urine of patients with tubular damage (13). Animal models have confirmed that it is one of the earliest detectable proteins in serum and urine in the case of ischemic kidney damage (14). After ischemia-reperfusion injury, the kidney responds within the first 30 minutes by releasing large amounts of NGAL into the urine through the epithelial cells of the tubules. Due to this fact, NGAL is also referred to as the "renal troponin," alluding to the role of troponin as a diagnostic and prognostic marker in acute coronary syndrome (15, 16).

In a prospective study by Kari et al. (17), a six–fold increase in NGAL in the urine of patients with AKI was observed two days before the rise in serum creatinine values. Sarafidis et al. (10) prospectively monitored the role of NGAL in detecting post–asphyctic AKI and found an increase in this marker compared to the control group on the first, third, and tenth day. It is particularly significant that the increase in NGAL was detected before the rise in serum creatinine values in these patients. Zhang et al. (18) even confirmed elevated NGAL values in the urine of neonates who experienced moderate intrauterine asphyxia, while serum creatinine and urea values were not elevated in these patients compared to the control group. The use of this marker can enable the early initiation of supportive therapy for these patients and improve survival prospects. Although numerous studies have shown that this biomarker has clinical utility in patients with post–asphyctic kidney damage, authors have not reached a consensus on the cutoff values for this parameter (19).

Kidney injury molecule 1 (KIM-1)

KIM-1 is a transmembrane glycoprotein with a mass of 104 kDa expressed by the proximal tubule cells of the kidney. Its particular significance lies in the fact that it cannot be detected under physiological conditions, and it is only excreted in urine after exposure of the kidneys to ischemia or toxins (20, 21). Previous research has shown that KIM-1 concentration in urine is highest in kidney damage resulting from ischemia, which is precisely the pathophysiological mechanism of renal damage in perinatal asphyxia. KIM-1 stands out from other biomarkers considering that it can be detected in urine as early as 5-6 hours after tubular cell damage (22-25). In a prospective study from 2022, Rumpel et al. (24) demonstrated that KIM-1 plays a significant role in predicting AKI in neonates diagnosed with HIE. In this study, KIM-1 had better predictive power compared to CysC and NGAL, but the authors note that, although statistically significant, KIM-1 has average specificity and sensitivity. Apart from this study, further data on the clinical utility of this marker in patients with PA are insufficient. However, there are studies on KIM-1 in the urine of pediatric patients with conditions that physiologically correspond to kidney damage in PA, i.e. renal ischemia. Assadi et al. (5) showed that urinary KIM-1 values can significantly contribute to the early detection of kidney damage in pediatric patients with circulatory collapse due to hypovolemic, cardiogenic, and distributive shock. A rapid test for detecting the ectodomain of KIM-1 in urine has been developed recently, requiring only 30 μL of the sam-

ple and providing results within 15 minutes, significantly facilitating its use in everyday clinical and biochemical practice (6).

Interleukin 18 (IL-18)

IL-18, formerly known as interferon-gamma-inducing factor, is a proinflammatory cytokine with a molecular mass of 18 kDa. Urinary IL-18 plays a crucial role in the inflammation of the renal interstitium, infiltration of neutrophil granulocytes and macrophages, and apoptosis of tubular cells (26). It represents an early, non–invasive biomarker of AKI. Its values are much more significant in acute kidney conditions compared to other renal diseases. As an early diagnostic marker, urinary IL-18 has sensitivity and specificity of over 90% (27, 28). It is synthesized in an inactive form and gets activated by the action of caspase-1 in the epithelial cells of proximal tubules. Its presence in urine indicates ischemic tubular necrosis, which has been confirmed in animal models. However, the exact mechanism of renal damage through the action of IL-18 is still insufficiently known (26).

In a prospective study from 2016, Oncel et al. (29) found that IL-18 values were significantly higher in neonates with asphyxia compared to the control group, as well as in asphyxiated neonates with AKI compared to asphyxiated neonates without AKI. In a sample of 105 neonates with perinatal asphyxia, Essajee et al. (30) found significantly higher values of urinary IL-18 in neonates with AKI. In a prospective study from 2022, Rumpel et al. (23) detected urinary IL-18 as a biomarker with predictive significance for the development of AKI among newborns with HIE undergoing therapeutic hypothermia. Apart from this study, it is not known that other authors have quantified IL-18 in the urine of neonates with HIE. The sample of interest in this population is mainly serum (31).

β2-Microglobulin (β2M)

β2M is a low-molecular-weight protein with a mass of 11.8 kDa that is filtered in the glomeruli of the kidneys and then completely absorbed by proximal tubular cells through endocytosis (32). Elevated levels of this biomarker are indicative of damage to proximal tubular cells. In cases of acute kidney injury due to ischemia, β2M can be detected in the urine within 48 hours of the onset of ischemia (32, 33). One limitation of this biomarker is that it degrades in acidic urine, so it is recommended that patients receive bicarbonates before quantifying this protein to raise the urine pH above 7 (34).

Research by El-Gendy et al. (35) suggests that urinary β2M levels serve as an indicator of renal insult with high specificity and sensitivity in neonates with perinatal asphyxia. In a prospective study with 80 term newborns with perinatal asphyxia, Abdullah et al. (36) identified β2M as an early biomarker of kidney damage. Mehrkash et al. (37) discovered significantly elevated levels of urinary β2M in neonates with perinatal asphyxia compared to the control group. The study also revealed significantly higher urinary β2M levels in neonates with perinatal asphyxia who developed acute kidney injury.

CONCLUSION

The mentioned biomarkers have demonstrated significance in individual studies, but further research in this field is necessary to confirm their use as clinically reliable indicators of kidney damage in newborns with diagnosed perinatal asphyxia. The absence of the exact "cut-off" values is making interpretation of results complicated, which is something that needs to be addressed in further research. Until then, a simultaneous use of multiple biomarkers could improve diagnostic sensitivity. Additionally, from a biochemical laboratory perspective, it is essential to find simpler and more accessible methods for their determination.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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Urinarni biomarkeri kao rani pokazatelji akutnog oštećenja bubrega kod novorođenčadi sa perinatalnom asfiksijom

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SAŽETAK

Uvod/Cilj. Perinatalna asfiksija (PA) predstavlja stanje u kojem je neposredno pre, u toku ili neposredno nakon porođaja smanjen ili prekinut dotok krvi i kiseonika u tkivu fetusa, tj. Novorođenčeta. Značajan je uzrok mortaliteta, i čini između 23% i 24% svih letalnih ishoda u neonatalnom periodu. Procenjena incidencija perinatalne hipoksije na globalnom nivou iznosi oko 0,5% od ukupnog broja živorođene dece gestacijske starosti preko 36 nedelja. PA ima negativan uticaj na čitav organizam, a posebno na tkiva koja su metabolički veoma zahtevna. Kako su bubrezi veoma osetljivi na deprivaciju kiseonika, akutno bubrežno oštećenje (ABO) može se razviti već u prva 24 sata od početka ishemične epizode. Ukoliko se ishemija prolongira, može doći i do ireverzibilne kortikalne nekroze. Rano prepoznavanje ABO-a veoma je važno radi adekvatne nadoknade tečnosti i elektrolita, budući da delovanje prerenalnih etioloških faktora predstavlja dinamičan proces čiji je početak reverzibilan. Ipak, ABO predstavlja loš prognostički znak. Mortalitet je veći kod neonatusa koji nakon perinatalne asfiksije razviju i ABO, a čak do 40% preživelih može imati trajno oštećenje bubrega. Imajući u vidu specifičnosti, kako populacije tako i samog kliničkog entiteta, jasna je potreba za novijim, senzitivnijim i specifičnijim biomarkerima bubrežne fukcije. Cilj ovog rada bio je pregled najznačajnijih urinarnih biomarkera kod novorođenčadi sa perinatalnom asfiksijom, koji bi mogli biti ključni za rano otkrivanje oštećenja bubrega.
Metode. Analizirani su naučni i stručni radovi objavljeni u poslednjih deset godina u međunarodnim naučnim i stručnim časopisima dostupnim u bazi podataka *PubMed*.
Zaključak. Vrsta biološkog uzorka, u kojem se potencijalni biohemijski marker kvantifikuje, ključna je karakteristika koja se mora uzeti u obzir. Za novorođenčad je od izuzetnog značaja da dobijanje uzorka bude neinvazivno. Imajući to u vidu, analiza urina se nameće kao dobar izbor. Metaboliti u urinu pacijenata sa PA dokazano su značajni za praćenje renalne funkcije. Nažalost, nedostatak urina kao biološkog uzorka ogleda se u nemogućnosti dobijanja uzorka odmah nakon rođenja, a značajan broj neonatusa usled već postojećeg renalnog oštećenja može biti anuričan.

Ključne reči: **perinatalna asfiksija, akutna bubrežna insuficijencija, urinarni biomarkeri**

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Review article

Up-to-date Modalities in the Prevention of Oral Mucositis: A Literature Review

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SUMMARY

Introduction/Aim. Oral mucositis is an acute, inflammatory, and ulcerative condition of the oral mucosa caused by chemotherapy and/or radiotherapy. Considering the frequency of oral mucositis, its impact on the physical and mental health of patients, as well as the depletion of the economic capacities of an individual and society, the importance of prevention and management of oral mucositis is clearly highlighted. The aim of our study was to determine the modern preventive modalities for oral mucositis.
Literature review. A search of studies indexed in the literature from 2002 to 2022 was conducted using the PubMed database. The search was conducted with the keywords: stomatitis, mucositis, oral mucositis, chemotherapy, radiotherapy, prevention, and oral cancer. There are numerous preventive modalities for oral mucositis, including: patient education, professional oral health care, home hygiene, rinsing solutions, anti-inflammatory agents such as benzydamine, photobiomodulation, cryotherapy, miconazole, liquid mucoadhesive hydrogel, high potency polymerized cross-linked sucralfate, morphine mouthwash solution, growth factors and cytokines, honey, vitamin C, vitamin E, vitamin B2, zinc, and glutamine.
Conclusion. The following preventive modalities for oral mucositis stand out as the most significant in the literature: benzydamine, laser therapy according to the specifications available in the literature, cryotherapy, 0.2% morphine mouthwash solution, and orally administered glutamine. The variability in the results indicates the complex nature of this clinical entity and the need for additional research, which will support the existing results and enrich the literature with new preventive modalities.

Keywords: **stomatitis, oral mucositis, chemotherapy, radiotherapy, prevention, oral cancer**

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INTRODUCTION

With the rise of chemotherapy as a therapeutic modality in the 1940s, the number of adverse changes of the oral mucosa, generally referred to as stomatitis, increased significantly. Due to the lack of effective therapeutic methods, as well as preventive guidelines for stomatitis, the quality of life and the prognosis of these patients have continuously worsened (1). In the past, the term "stomatitis", in addition to being used for the changes that occur because of chemotherapy and/or radiotherapy, was also used for many other diseases affecting the oral mucosa. The term "mucositis" i.e., "oral mucositis" began to be used in the 1980s as a more precise term describing lesions resulting from cytotoxic cancer therapy (2). The complex pathogenetic mechanisms of the previously called stomatitis were discovered in 2007, and thus the new term "oral mucositis" was officially adopted, which described the lesions that occur because of the cytotoxic effects of chemo- and/or radiotherapy. In the same year, ICD-9 code 528.0 was assigned to lesions associated with cytotoxic cancer therapy. The ICD-10 code for oral mucositis is K12.3 (3).

Oral mucositis is an acute, inflammatory, and ulcerative condition of the oral mucosa whose incidence during chemotherapy is 40%, and in combination with radiotherapy, the incidence rate reaches a value close to 100% (4, 5). Depending on the intensity of the changes of oral mucositis, there may be a significant decrease in the quality of life of these patients due to disturbance of nutrition and sleep, communication problems, and immense pain (6). In certain cases, patients may lose consciousness because of pain and dehydration, which further necessitates stopping cancer treatment (7).

Considering the frequency of oral mucositis, its impact on the physical and mental health of patients, as well as the depletion of the economic capacities of an individual and society, the importance of prevention and management of oral mucositis is clearly highlighted (8). Since the literature indicates different preventive modalities for this clinical entity (9-18), the aim of our study was to determine the modern preventive modalities for oral mucositis.

LITERATURE REVIEW

A search of studies indexed in the literature from 2002 to 2022 was conducted using the PubMed database. The search was conducted with the keywords: stomatitis, mucositis, oral mucositis, chemotherapy, radiotherapy, prevention, and oral cancer. The data were grouped according to a frequently used division of preventive modalities for oral mucositis (19-22): 1) Basic oral hygiene; 2) Anti-inflammatory therapy; 3) Photobiomodulation; 4) Cryotherapy; 5) Antimicrobial agents, coating agents, anesthetics, and analgesics; 6) Growth factors and cytokines, and 7) Natural and miscellaneous agents. We used the additional keywords (23) for each of the separate categories: basic oral care, chlorhexidine, patient education, anti-inflammatory agents, laser therapy, low-level laser therapy (LLLT), photobiomodulation, cryotherapy, analgesics, antimicrobials, mucosal coating agents, growth factors, cytokines, natural products, honey, aloe vera, vitamin E, vitamin C, vitamin B, zinc, and glutamine. We included English-language sources that, with appropriate clinical, histological, or molecular data, evaluate the effect of the appropriate preventive agent for oral mucositis.

After searching the literature, a total of 90 sources were used for this study, including: original papers, literature reviews, systematic reviews, clinical studies, randomized controlled studies, and books.

According to the available data, the division of the different preventive modalities we used was initially introduced by the Multinational Association of Supportive Care in Cancer and International Society of Oral Oncology (MASCC/ISOO), which have a great contribution for the prevention and management of oral mucositis and which, in the period from 2004 to 2021, are constantly researching and renewing clinical guidelines for the prevention and treatment of chemotherapy-induced oral mucositis (19-23).

Basic oral care

When it comes to basic oral care, it is inevitable to mention patient education, professional oral

health care, as well as home hygiene and the use of different solutions for rinsing the oral cavity (24). Several studies evaluating the effect of patient education as a preventive measure for oral mucositis were available in the recent literature (25-28). The studies implement training sessions for self-assessment and maintenance of oral health with professional staff. Three studies (25, 27, 28) found that patient education significantly reduced the frequency and intensity of oral mucositis in patients with head and neck cancer and patients with hematologic-associated cancer, while a comparative study by Schmidt et al. (26) found no benefit from patient education. We believe that patient education is undoubtedly useful and may result in patient benefit despite the diversity of data from the literature.

The impact of professional oral health care in relation to oral mucositis is usually evaluated by evaluating the intensity of oral mucositis and the intensity of pain. In two studies (29, 30), a reduction in the intensity of oral mucositis was registered, while one study (31) registered a reduction in pain as a result of oral mucositis during regular implementation of professional oral health care. Dental evaluation and treatment as indicated prior to cancer therapy are desirable to reduce the risk of local and systemic infections from odontogenic sources.

Considering chlorhexidine as a rinsing solution, the available data was consistent (22, 32, 33) and indicates that chlorhexidine does not have a preventive effect on the occurrence of oral mucositis. However, this does not preclude other indications for chlorhexidine in cancer patients, such as prevention or treatment of oral infections. If chlorhexidine is indicated because of a concomitant oral infection and oral mucositis, it is acceptable to use it because of the oral infection.

Anti-inflammatory agents

When it comes to anti-inflammatory therapy and prevention of oral mucositis, the use of benzydamine solution for rinsing the oral cavity is most often mentioned in the literature. The results of numerous studies investigating the effect of benzydamine on the severity of oral mucositis are summarized in the studies of Ariyawardana et al. (34) and Nicolatou-Gallitis et al. (35) and indicate a significant reduction in the intensity of oral mucositis and pain in patients receiving chemo- and/or radiotherapy. Benzydamine exhibits anti-inflammatory properties by inhibiting the production of pro-inflammatory cytokines such as TNFα and IL-1β (36) which play a key role in the pathogenetic mechanisms of oral mucositis (34, 37).

Photobiomodulation

Recent data from the literature support the use of photobiomodulation for the prevention of oral mucositis, especially in bone marrow transplantation, in head and neck radiotherapy (without chemotherapy) and in head and neck radiotherapy in combination with chemotherapy (38). To achieve an optimal therapeutic effect, it is important to precisely follow the recommendations and specific settings of the laser published in the literature, which depend on the reason for therapy, as well as the type of therapy (radiotherapy or a combination of radio- and chemotherapy) (22). Bensadoun and Nair (39) recommend the use of red or infrared LLLT with diode output between 10-100 mW, dose of 2-3 J/cm^2/cm^2 for prophylaxis and 4 J/cm^2 (maximum limit) for therapeutic effect, application on single spot rather than scanning motion.

Cryotherapy

Conventional methods for applying cryotherapy in the oral cavity are the use of cold water or ice, but there are other, newer methods, and commercial devices that are used in daily practice (40). Vasoconstriction caused by low temperatures reduces the transport of cytotoxic drugs to oral tissues and thus prevents secondary complications (41). Additionally, low temperatures reduce metabolic activity in the basal layer, making the epithelium less sensitive to cytotoxic agents (42).

The effects of cryotherapy have been investigated in bone marrow transplant patients (43-48), in patients with 5-FU bolus chemotherapy for solid tumors (49, 50), in patients treated with short-term infusion chemotherapy and short-half-life agents (41), and in patients treated with head and neck radiotherapy (51).

The latest guidelines recommend the use of cryotherapy in two cases: in patients undergoing autologous bone marrow transplantation treated with high doses of melphalan; in patients receiving 5-FU bolus therapy, 30 minutes during the therapy itself (22).

Antimicrobials, coating agents, anesthetics, and analgetics

Candidiasis is a common oral infection in patients with cancer and it can secondarily infect the lesions of oral mucositis, worsening the symptoms and making it difficult for them to epithelize. Therefore, it is theorized that antifungal drugs can prevent oral mucositis (52).

Rao et al. (53) in their study of 181 patients registered a reduced incidence of oral mucositis and oral candidiasis with twice weekly prophylactic administration of fluconazole during chemoradiotherapy in patients with head and neck cancer.

Miconazole is a synthetic imidazole antifungal agent that is often used to treat candidomycotic infections. However, oral medications for topical use, due to dynamics in the oral medium, need to be applied frequently, which makes it difficult for patients to cooperate and adhere to the therapeutic regimen. Therefore, Orvain et al. (54) in their study investigated the new formulation of miconazole, administered as a mucoadhesive buccal tablet, but the research was not aimed at monitoring oral mucositis, but at indirect indicators (hospitalization time, morphine use) of oral mucositis.

The oral mucosa of cancer patients is more susceptible to physiological trauma. For this purpose, coating agents have been created that form a barrier that reduces the irritation of the oral mucosa (52). Several studies of viscous liquid mucoadhesive hydrogel (MAH) are found in the literature (55-57), however, the results of these studies are not sufficient to establish official guidelines for the use of this preparation for the prevention of oral mucositis. Complete prevention and rapid elimination of oral mucositis were registered in McCullough's research (58), which opens new directions for studying the coating agent used in the study—high-potency polymerized cross-linked sucralfate (HPPCLS).

From the category of analgesics, the local use of 0.2% morphine mouthwash solution is recommended for the regulation of pain caused by oral mucositis in patients with head and neck cancer treated with chemoradiotherapy (22, 59, 60).

Growth factors and cytokines

Growth factors and cytokines can stimulate the regeneration of oral mucosa cells, preventing oral mucositis and reducing its negative effects (61). The effects of different growth factors and cytokines have been studied in the literature, such as: granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF) (62-64), epidermal growth factor (EGF) (65, 66), erythropoietin (EPO) (67), as well as the most frequently mentioned family of growth factors, which give the most favorable results keratinocyte growth factors (KGF) in the form of palifermin (68-72).

The most recent guidelines recommend the use of intravenous KGF-1 for the prevention of oral mucositis in patients with hematologic malignancies who have undergone bone marrow transplantation (22, 73). Current data do not recommend the topical use of GM-CSF for the prevention of oral mucositis in patients with hematological cancer who have undergone bone marrow transplantation (22, 74).

Natural and miscellaneous agents

Honey has often been investigated in medicine, due to its: antioxidant, anti-inflammatory, antibacterial, antiviral, antifungal, antitumor, antimutagenic, and regenerative properties (75-79).

Charalambous et al. (76) evaluated and determined the potential effect of a solution of thyme and honey to improve quality of life and improve symptoms in patients with head and neck cancer. According to Khanjani et al. (80), the use of an aqueous solution of honey (in the ratio of honey: water, 1:20) is effective for the prevention and reduction of the intensity of oral mucositis in patients with acute myeloid leukemia. Sener et al. (81) treated patients with oral mucositis with a mixture of honey and vitamin E and found that this solution better controlled oral mucositis than chlorhexidine. According to the latest guidelines (22), honey is recommended for the prevention of oral mucositis in patients with head and neck cancer treated with radiotherapy or chemoradiotherapy, but honey also has a cariogenic effect, so its application must be moderate (82).

The most common form of vitamin E—α-tocopherol has cytoprotective and anti-inflammatory characteristics (83). The efficacy of vitamin E for the prevention and regulation of oral mucositis has been investigated in different tumors/carcinomas, where it has been administered in different forms: solution for gargling and swallowing in hematological patients treated with chemotherapy (84), tablet form, and as an oil in hematological patients and patients

treated with chemotherapy (85), topical use in solid tumors treated with chemotherapy (86, 87) and in the form of a solution for gargling and swallowing in patients with head and neck cancer treated with radiotherapy (88).

Ferreira et al. (89) evaluated the prophylactic efficacy of vitamin C and vitamin B2 on methotrexate-induced gastrointestinal mucositis in an animal model. The authors (89) registered a benefit from the use of vitamin C but not from the use of vitamin B2. Rasheed et al. (90) examined the concentration of vitamin C in bone marrow transplant patients and registered a more advanced form of mucositis in patients with a lower concentration of vitamin C in the body. Kletzel et al. (91) prescribed vitamin C (2 g per day) in bone marrow transplant patients and detected an improvement in the clinical manifestation of oral mucositis and an improvement in quality of life through a pain-free diet.

In the literature, when it comes to the prevention of oral mucositis, zinc is often mentioned as an important electrolyte for the homeostasis of the body, which is involved in the processes of wound healing and the immune response of the individual (86). Chaitanya et al. (92) found in their research that zinc treatment resulted in a milder clinical presentation of oral mucositis, and vitamin C treatment resulted in less pain in the subjects. In combination, zinc and vitamin C resulted in milder clinical manifestation, less pain and better taste perception. Other studies also confirm the efficacy of zinc in the prevention of oral mucositis (93-95), however, there are also studies that indicate the absence of efficacy of zinc in the prevention of oral mucositis (96, 97). Due to the diversity of results, there are still no official guidelines for the use of zinc in the prevention of oral mucositis.

Due to the protective effect of saliva, different methods of stimulation of salivary secretion and their effect on the prevention of oral mucositis were investigated in the literature, such as: stimulation with chewing gum (98, 99), electrical stimulation (100), and intravenous application of N-acetyl cysteine (101). There is not enough evidence about the preventive effect of the mentioned methods (98-101) in the prevention of oral mucositis.

Glutamine is an amino acid that is present in large quantities in blood plasma and plays a significant role in cell survival under conditions of metabolic stress (102). Studies in the literature evaluating the preventive effect of glutamine on oral mucositis examined it in two different forms: parenterally and per os (103-108). Considering the studies in which there is an absence of evidence of benefit from parenteral administration of glutamine, as well as one study (103) in which a higher mortality rate was recorded in cancer patients who were given parenteral glutamine, there is no support that the parenteral form of this preparation should be used for the prevention of oral mucositis. Several studies show a positive effect of orally (per os) administered glutamine in patients treated with chemo- and chemoradiotherapy (109-112), which is the supported way of using glutamine for the prevention of oral mucositis (22).

CONCLUSION

Through this research and critical evaluation of the available literature related to oral mucositis, we can note that there is considerable variation in the preventive power of different agents. Undoubtedly, patient education and regular consultations with the dentist are important for the prevention of oral mucositis, but we can single out the following agents and preventive modalities as equally important: benzydamine, laser therapy according to the specifications available in the literature, cryotherapy, 0.2% morphine mouthwash solution, and orally administered glutamine.

The variability in the results indicates the complex nature of this clinical entity and the need for additional research, which will not only support the results of previous research but also enrich the literature with new possibilities for the prevention of this complication caused by chemo- and/or radiotherapy.

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Savremeni modaliteti u prevenciji oralnog mukozitisa: pregled literature

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SAŽETAK

Uvod/Cilj. Oralni mukozitis predstavlja akutno, inflamatorno i ulcerozno stanje oralne sluzokože, koje je uzrokovano hemioterapijom i/ili radioterapijom. S obzirom na učestalost oralnih mukozitisa, njihov uticaj na fizičko i mentalno zdravlje pacijenata, kao i na crpljenje ekonomskih kapaciteta pojedinaca i društva, jasno je naglašen značaj prevencije i lečenja mukozitisa. Cilj našeg istraživanja bio je da se utvrde savremeni modaliteti u prevenciji oralnih mukozitisa.

Pregled literature. Pretraživanje indeksiranih studija od 2002. do 2022. godine sprovedeno je korišćenjem baze podataka *PubMed*. Pretraga je vršena prema sledećim ključnim rečima: stomatitis, mukozitis, oralni mukozitis, hemioterapija, radioterapija, prevencija i oralni karcinom. Postoje brojni preventivni modaliteti za oralni mukozitis, koji uključuju: edukaciju pacijenata, profesionalnu oralnu zdravstvenu zaštitu, kućnu higijenu, rastvore za ispiranje usta, antiinflamatorna sredstva poput benzidiamina, fotobiomodulaciju, krioterapiju, mikonazol, tečni mukoadhezivni hidrogel, polimerizovani visokopotentni umreženi sukralfat, rastvor morfijuma za ispiranje usta, faktore rasta i citokine, med, vitamin C, vitamin E, vitamin B2, cink i glutamin.

Zaključak. U literaturi se kao najznačajniji izdvajaju sledeći modaliteti prevencije oralnih mukozitisa: benzidiamin, terapija laserom prema uputstvima dostupnim u literaturi, krioterapija, 0,2% rastvor tečnosti za ispiranje usta na bazi morfijuma i oralno primenjeni glutamin. Varijabilnost u rezultatima ukazuje na kompleksnu prirodu ovog kliničkog entiteta i potrebu za dodatnim istraživanjima koja će potkrepiti postojeće rezultate i obogatiti literaturu novim preventivnim pristupima.

Ključne reči: **stomatitis, oralni mukozitis, hemioterapija, radioterapija, prevencija, rak usne duplje**

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Review article

The Latest Recommendations in the Prophylaxis and Treatment of Bleeding from Esophagogastric Varices

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SUMMARY

Introduction/Aim. Esophagogastric varices develop in 50-60% of patients with liver cirrhosis, and 30% of them have one episode of variceal hemorrhage within two years of variceal diagnosis. The aim of the paper was to present the latest attitudes in the treatment of esophagogastric varices.
Literature review. Prevention of first bleeding from esophageal varices (EV) involves the use of non-selective beta blockers (NSBB) or carvedilol, while in case of their intolerance or contraindications for their use, endoscopic band ligation (EBL) should be performed. In acute variceal bleeding, endoscopy should be performed, preferably within 12 hours of the presentation of the bleeding, and EBL should be applied. In case of refractory hemorrhage (about 20%), repeated endoscopy and hemostasis or balloon tamponade, self-expanding metal stent (SEMS), transjugular intrahepatic portosystemic shunt (TIPS) and surgical therapy are required. Bleeding from gastric varices (GV) is less common than bleeding from EV but is significantly more severe with higher mortality and more frequent treatment failure. The therapy of choice is the application of cyanoacrylate (CYA), which can be applied under endoscopic ultrasonography (EUS) control. In the trial is the administration of coil injections with or without CYA. In the secondary prophylaxis of bleeding from EV, NSBB should be used in combination with EBL. In the secondary prophylaxis of bleeding from cardiofundal varices, the approach is individual.
Conclusion. The therapy of choice for the primary prevention of bleeding from EV is NSBB, while the combined therapy (NSBB and EBL) is for the secondary prophylaxis of bleeding. CYA is the therapy of choice for GI bleeding. Refractory variceal hemorrhage requires the application of many therapeutic modalities.

Keywords: **esophagogastric varices, prophylaxis, treatment**

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INTRODUCTION

Portal hypertension (PH) develops as a consequence of increased portal flow resistance, which is also contributed to by an increase in collateral portal blood flow resistance. The obstruction of portal flow can be at different levels: pre-sinusoidal (e.g., due to schistosomiasis, portal vein thrombosis); sinusoidal (for example, advanced chronic liver disease); post-sinusoidal (e.g., Budd-Chiari syndrome) (1).

Although patients with cirrhosis and portal hypertension may bleed at various sites, ruptured esophagogastric varices are the most severe and common cause of gastrointestinal (GI) bleeding, accounting for nearly 80% of bleeding episodes in these patients. Moreover, about 60-80% of bleeding in patients with liver cirrhosis is from esophageal varices (EV), and about 7% from gastric varices (GV). Varicose veins develop in 50–60% of patients with liver cirrhosis, and 30% of them have one episode of variceal hemorrhage within two years of variceal diagnosis. Variceal bleeding accounts for 2–20% of all GI bleeding and 50% of severe, persistent bleeding. The greatest risk of bleeding from varices is within 6–12 months from their discovery.

About 5–8% of patients die within 24 hours due to uncontrolled variceal bleeding. Significant prognostic indicators of inability to control variceal bleeding are: active bleeding during emergency endoscopy, bacterial infection, and portosystemic pressure gradient greater than 20 mmHg. The mentioned factors, together with low serum albumin values and kidney failure, are significant prognostic indicators of the risk of early rebleeding from varices. After the initial bleeding, the incidence of early rebleeding within the first six weeks varies from 30–40%. The greatest risk is within the first five days, during which 40% of all rebleeding episodes occur (2, 3).

Mortality from variceal bleeding is estimated at six weeks. Earlier studies showed that the mortality was 30–50%. However, with the development of more effective therapeutic measures, mortality has fallen to 15–20% today. Very important prognostic indicators of the risk of death are the severity of liver disease, renal insufficiency, persistent variceal bleeding, and recurrent bleeding (4).

The aim of this review paper was to show the latest recommendations regarding the primary prophylaxis of bleeding from esophagogastric varices (in patients who had not had previous bleeding from varices), treatment of acute bleeding and secondary prophylaxis of variceal bleeding, i.e., prevention of rebleeding in patients who had survived the first episode of bleeding.

DIAGNOSIS OF ESOPHAGOGASTRIC VARICES AND RISK STRATIFICATION

The gold standard in the diagnosis of esophagogastric varices is esophagogastroduodenoscopy (EGD), which, in addition to diagnosing varices, also stratifies the risk of bleeding from varices based on their size and high-risk stigmata. EV are classified according to size into small, medium, and large, with or without the presence of risk signs of bleeding in the form of various forms of red spots (1). GV typically occur in the advanced stage of portal hypertension. Sarin's classification of GV includes four types of varices: gastroesophageal varices type 1 (GOV1) are the most common (74%), they extend 2 to 5 cm below the gastroesophageal junction and are continuous with the EV; gastroesophageal varices type 2 (GOV2) are in the cardia and fundus of the stomach and are in continuity with the EV; isolated GV type 1 (IGV1) are varices that occur in the fundus of the stomach in the absence of EV; isolated GV type 2 (IGV2) occur in the body of the stomach, antrum or pylorus (Figure 1) (5). The risk factors for bleeding from GV are: 1. Localization of varices—bleeding is more common in GOV2 and IGV1, which are usually called "cardiofundal varices", than in the other two types of GV; 2. Varicose size—larger veins (> 20 mm) bleed more often than smaller ones; 3. The presence of risk signs or the so-called "red spots" on the varicose veins; 4. Severity of liver disease—MELD score over 17 (3, 6).

Hepatic venous pressure gradient (HVPG) is the gold standard for assessing clinically significant portal hypertension that is present if values are greater than 10 mmHg (7). In practice, non-invasive tests are increasingly used to assess clinically significant portal hypertension, such as the assessment of liver fibrosis by elastography, platelet count, and spleen size (8-10). According to the Baveno VII consensus, EGD is not necessary as a screening for varices if liver fibrosis values on elastography are less than 20 Kpa and the platelet count is more than 150 x 109/L, because these values indicate a very low probability (< 5%) that the patient has high risky varicose veins. If a patient with diagnosed liver cirrhosis does not meet these criteria, endoscopic screening for varices is recommended (11).

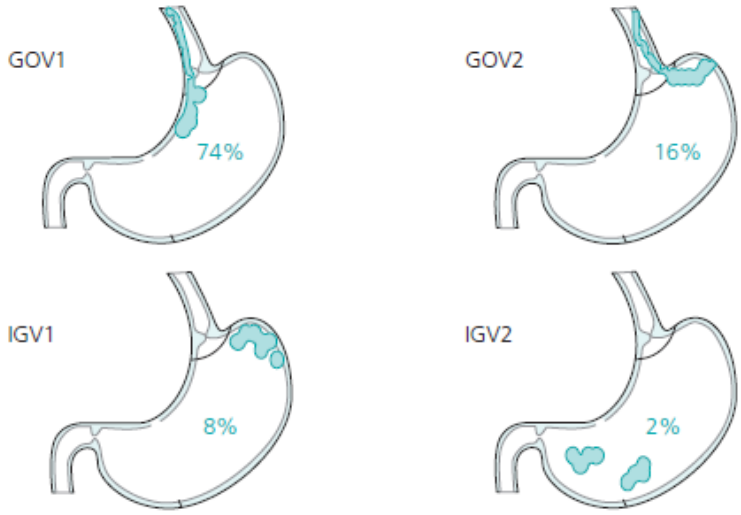

Figure 1. *Different types of gastric varices according to Sarin's classification (GOV, gastroesophageal varices; IGV, isolated gastric varices) (5)*

PRIMARY PROPHYLAXIS OF VARICEAL BLEEDING

The indication for primary prophylaxis of variceal hemorrhage is an advanced chronic liver disease (ACLD) and endoscopically diagnosed high-risk varices. Non-selective beta blockers (NSBB) or endoscopic band ligation (EBL) significantly reduce the risk of a first episode of variceal bleeding (1).

According to the recommendations of the European Society for GI Endoscopy (ESGE), patients with compensated ACLD (caused by viruses, alcohol and/or non-alcoholic steatohepatitis in non-obese population with BMI < 30 kg/m^2) and clinically significant portal hypertension with HVPG > 10 mmHg and/or liver fibrosis on elastrography > 25 Kpa should be on NSBB, primarily carvedilol, for the prevention of variceal bleeding. Screening endoscopy is not necessary in patients with compensated liver cirrhosis who use NSBB in the primary prophylaxis of variceal hemorrhage. In case of intolerance to NSBB or contraindications for their use, EBL is indicated as the therapy of choice. EBL should be repeated every 2-4 weeks until the complete eradication of the varix. EGD should be repeated every 3-6 months in the first year after varix eradication (strong recommendation, medium level of evidence) (12). Similar recommendations were given in the Baveno VII consensus, according to which if ascites and low-risk small varices (< 5 mm), as well as high-risk large varices, are present, the therapeutic choice is NSBB or carvedilol. Dose reduction or discontinuation of NSBB and carvedilol is required when systolic blood pressure falls below 90 mmHg, mean arterial pressure below 65 mmHg and/or the development of hepatorenal syndrome. After stabilization of these parameters, NSBB or carvedilol can be reintroduced into therapy. Also, the recommendation for the use of EBL in the primary prophylaxis of variceal hemorrhage is only for intolerance to NSBB (11). This position has been significantly modified in relation to the Baveno VI consensus recommendations (13).

Comparing the effect of NSBB and EBL, the studies have showed that side effects are more common with EBL, but discontinuation due to intolerance is more common with NSBB. The benefit in terms of survival is greater with NSBB compared to EBL, which is most likely due to the effect of reducing portal pressure. The efficiency of NSBB and EBL in reducing the incidence of first bleeding from EV is similar (12). Thus, meta-analysis by Sharma et al. (14) showed similar efficiency of NSBB and EBL in reducing the risk of first variceal bleeding. This analysis included 3,362 patients with liver cirrhosis and large EV. Another meta-analysis by Villanueva et al. (15) that included 11 randomized controlled trials (RCT) showed that the risk of mortality was lower in the group treated with NSBB than in the group of patients treated with EBL (p = 0.02), probably as a consequence reducing the risk of ascites. The risk of first variceal bleeding was similar be-

tween the treated groups of patients (p = 0.86). According to other authors, there was no difference in terms of mortality when using EBL and NSBB (16).

The advantage of carvedilol over classic NSBB in the primary prophylaxis of variceal bleeding is that it leads to a greater reduction in portal pressure, but there are not enough randomized studies that would deal with the comparative analysis of carvedilol and NSBB. A study by Reiberger et al. (17) showed that the use of carvedilol in primary prophylaxis in patients who did not respond to propranolol achieved hemodynamic response, which led to improved outcomes in terms of prevention of variceal bleeding, hepatic decompensation, and death. A recent meta-analysis by Tian et al. (18) compared the effect of carvedilol and EBL in the primary prophylaxis of variceal bleeding and found no significant differences in terms of variceal bleeding, mortality, and especially mortality related to variceal bleeding.

Primary prophylaxis of bleeding from GV

In the primary prophylaxis of GV bleeding, ESGE recommends to patients with Sarin GOV2 and IGV1, who do not tolerate NSBB, the option of only observation without treatment, injection of cyanoacrylate (CYA) or endoscopic ultrasound-guided coil therapy with CYA in centers experienced in the application of this technique (weak recommendation, low level of evidence) (12). CYA was shown to be more effective than propranolol in preventing the first bleeding from large GOV2 and IGV; however, there was no difference in survival. There are no indications for the use of balloon-occluded retrograde transvenous obliteration (BRTO) or transjugular intrahepatic portosystemic shunt (TIPS) for primary prophylaxis of GV bleeding (1).

TREATMENT OF ACUTE VARICEAL BLEEDING

Hemodynamic resuscitation

Rupture of esophagogastric varices presents with severe hemorrhage, i.e., hematemesis and/or melena, severe anemia and possible confusion of consciousness. This requires urgent patient care in the intensive care unit. Initially, the patient should be hemodynamically stabilized in order to improve tissue perfusion, correct intravascular hypovolemia and prevent multiorgan dysfunction. Crystalloid solutions in limited quantities are recommended, which reduce mortality and adverse renal effects compared with saline (19). According to the Baveno VII consensus, red blood cell transfusions should achieve hemoglobin target values of 7-8 g/dl, although other factors such as cardiovascular disorders, age, hemodynamic status and bleeding intensity should be taken into account when assessing hemoglobin target values. Intubation of the patient is indicated before endoscopy in patients with impaired consciousness and active blood vomiting. Extubation should be done as soon as possible after endoscopy (11). In the event of suspected variceal bleeding in patients who are on antiplatelet and anticoagulant therapy, the attitude regarding the discontinuation of this therapy is based on the assessment of the risk of bleeding and thrombosis. According to recently published British Society for Gastroenterology (BSG) and European Society for Gastrointestinal Endoscopy (ESGE) guidelines, aspirin should be discontinued and not reintroduced if given as primary prophylaxis (12, 20, 21). If aspirin is given as secondary cardiovascular prophylaxis, reintroduction of aspirin should be considered in the context of assessing the risk of variceal rebleeding and the risk of thrombosis.

It should be noted that the restoration of normal platelet function after discontinuation of aspirin occurs minimally after 5–7 days. P2Y12 receptor antagonists in patients with coronary artery stents should be returned to therapy within five days because of the high risk of stent occlusion (22).

Vasoactive drugs

In suspected variceal hemorrhage, vasoactive drugs, such as terlipressin or octreotide, should be started as soon as possible and continued for 2-5 days (11). According to some studies that evaluated the effectiveness and safety of vasoactive drugs in acute variceal bleeding, the use of these drugs affected the reduction of in-hospital mortality, overall mortality, better control of variceal bleeding, reduction of variceal rebleeding and reduction of the need for blood transfusions. Octreotide is as effective as terlipressin and vasopressin, but with fewer side effects, especially compared to vasopressin (1, 23, 24).

Antibiotic prophylaxis

Patients with acute variceal bleeding are at high risk of bacterial infection. According to ESGE recommendations, antibiotic prophylaxis with ceftriaxone 1 g per day for up to 7 days is indicated, which implies knowledge of local antibiotic resistance and the patient's possible allergy to this drug (12). The use of ceftriaxone is especially recommended for patients with quinolone-resistant bacterial infections and patients who have previously received quinolones. Bacterial infections lead to an increased risk of varices rebleeding and increase overall mortality. Despite antibiotic prophylaxis, 14% of patients develop bacterial infections, mostly respiratory, within 14 days of bleeding (25). The risk of bacterial infection is very low in patients with Child-Pugh A liver cirrhosis. Chang et al. (26) showed that the incidence of bacterial infection within 14 days and overall mortality within 42 days were not different in patients with Child-Pugh stage A cirrhosis who received antibiotics prophylactically and who received them on an as-needed basis. However, more prospective studies are needed before concluding that antibiotic prophylaxis is not necessary in this subgroup of patients.

Timing of upper gastrointestinal endoscopy

According to ESGE recommendations, in case of suspected variceal hemorrhage, endoscopy should be performed within 12 hours of the presentation of bleeding in a hemodynamically stabilized patient (12). In case of impossibility of hemodynamic stabilization, endoscopy should be performed as soon as possible (11). A systematic review and meta-analysis by Bai et al. (27) on 2,824 patients showed that overall mortality was significantly lower in early endoscopy (within 12 h) compared to delayed endoscopy (after 12 h).

Endoscopic treatment of acute variceal bleeding

A strict recommendation with a high quality of evidence by the ESGE is for the use of EBL in acute variceal bleeding (12). Varicose strangulation, thrombosis, and obliteration are achieved with ligatures. Rings are placed first on varices with signs of recent bleeding or active bleeding. It usually starts from the esophagogastric junction and spirals proximally for about 2 cm until all varices are ligated (Figure 2 and 3). The interval between ligation sessions is usually 14 days until the varices are completely obliterated or their size reduced to the first degree. After the eradication of the varix, control endoscopies are performed every 3-6 months. With EBL, control of active variceal bleeding is achieved in about 90% of cases (1, 28, 29). Meta-analyses have shown that EBL is superior to endoscopic variceal sclerotherapy (EVS) in terms of rebleeding, complications, and eradication of varices, however, there was no difference in mortality (30). Nevertheless, EBL is associated with a higher incidence of variceal recurrence because obliteration of paraesophageal varices is not possible. Therefore, in many studies, the simultaneous application of EBL and EVS was attempted, but no benefit of the combined therapy was shown, and the participation of complications was increased. Therefore, the conclusion is that simultaneous combined therapy of EBL and EVS is not recommended (31–33). Another approach to the combined therapy of EBL and EVS is the application of a smaller amount of sclerosing agent after size reduction of the varix using EBL. Fewer recurrences of varices would be expected considering that paraesophageal varices are obliterated by sclerotherapy. Also, the use of a smaller amount of sclerosing agent should reduce the frequency of sclerotherapy complications. Thus, according to some studies, EVS can be beneficial if applied to very small varices left after EBL (34–36). Our study showed that in the group of the combination of ligatures and sclerotherapy, there was less recurrence of varices compared to ligation alone (16% vs. 21.7%, respectively), but the difference was not statistically significant. Rebleeding from varices was identical in both groups of patients. The conclusion of our study was that combined therapy has no advantage over EBL alone (37).

Complications after ligation of varices are less frequent and easier than after sclerotherapy. Chest pain and dysphagia after ligation are transient. After sclerotherapy, there are numerous complications: dysphagia, chest pain, feverishness, pleural effusions, ulcers, and esophageal strictures (38).

Hemostatic spray/powder has recently been introduced in the treatment of GI bleeding, primarily bleeding from ulcers and tumors. It is applied through a special catheter. Hemospray is an inert mineral-based powder that absorbs water in contact with blood and adheres to the damaged area. Ac-

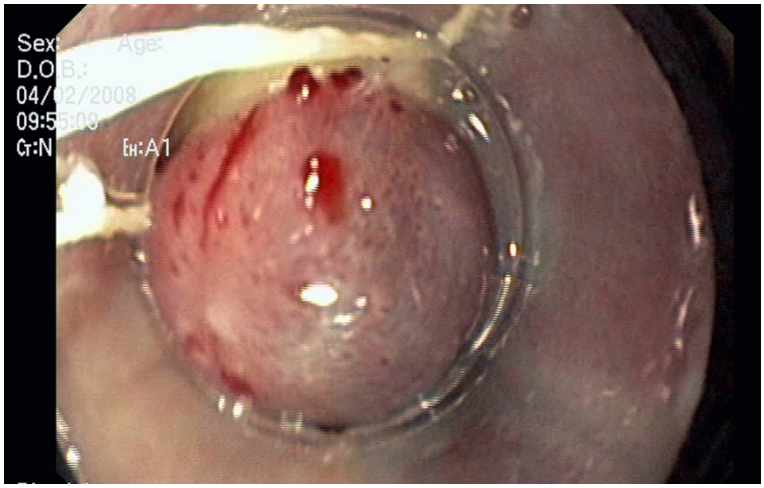

Figure 2. *Suction of the esophageal mucosa, submucosa, and the varix (Grgov S)*

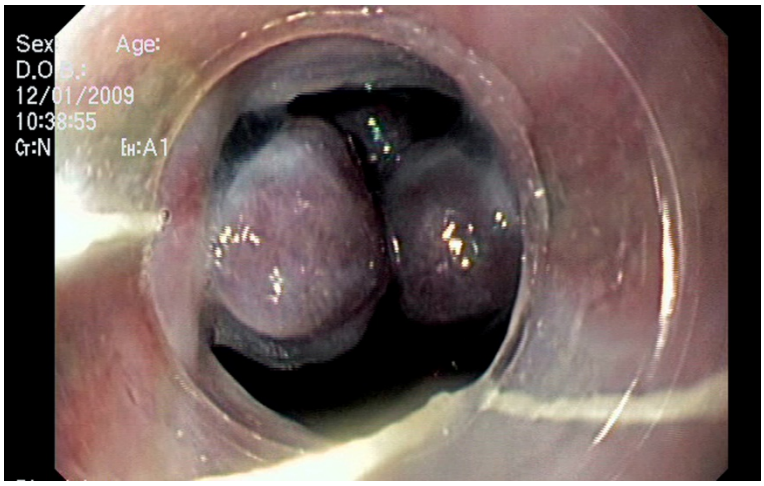

Figure 3. *Three ligated varices at the bottom of the esophagus (Grgov S)*

cording to ESGE recommendations, Hemospray can be used as a bridge to definitive therapy of bleeding from varices (12).This therapy can stabilize a patient with variceal bleeding until definitive endoscopic treatment. For now, the application of hemostatic powders cannot be recommended as the first line of endoscopic therapy due to the lack of evidence of benefit (11).

TREATMENT OF REFRACTORY VARICEAL HEMORRHAGE

Up to 20% of variceal hemorrhage can be refractory to standard therapy due to massive bleeding, inability to establish endoscopic hemostasis or rapid onset of rebleeding (1). Mortality in such cases is 30–50% (39). There are several therapeutic options: repeat endoscopy and hemostasis, balloon tamponade, self-expanding metal stent (SEMS), TIPS, and surgical therapy (Figure 4).

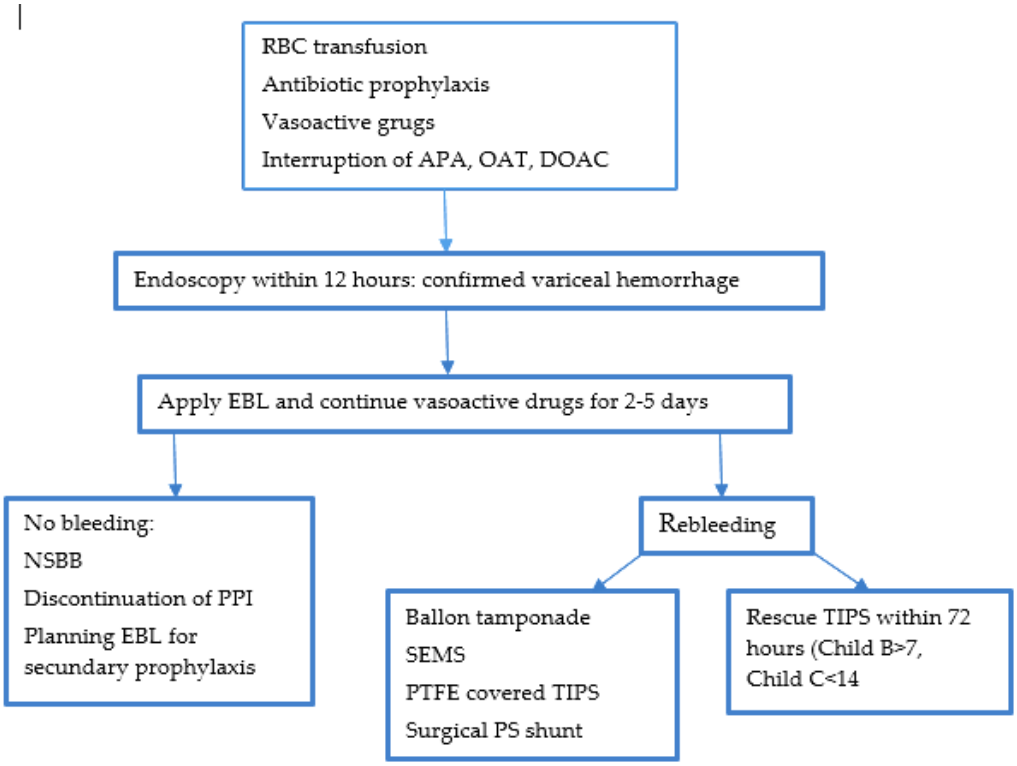

Figure 4. *Suspected variceal bleeding (RBC– rubber blood cell; APA–antiplatelet agents; OAT–oral anticoagulant therapy; DOAC–direct oral anticoagulant; EBL–endoscopic band ligation; NSBB–non-selective beta blocker; PPI–proton pump inhibitor; SEMS–self expanding metallic stent; PTFE–covered TIPS, polytetrafluoroethylene-covered TIPS; PS–potosystemic*

Balloon tamponade

Balloon tamponade involves the use of a Sengstaken-Blakemore or Minnesota probe. It represents an effective temporary measure in case of failure of endoscopic hemostasis or impossibility of applying endoscopic hemostasis. Balloon tamponade controls bleeding in about 80% of cases. Adverse effects can be serious, such as esophageal ulceration, esophageal perforation, or aspiration pneumonia in up to 20% of patients (3). Balloon tamponade should be stopped in no more than 24 hours, but the rate of rebleeding after removal of balloon tamponade is about 50% (12).

Self-expanding metal stent (SEMS)

The ESGE recommendation for persistent variceal hemorrhage despite the use of vasoactive drugs and endoscopic hemostasis is the use of SEMS, rather than balloon tamponade. The stent can remain in the esophagus for up to 14 days allowing definitive treatment to be planned. Possible side effects are stent migration and ulceration. SEMS is more expensive option compared to balloon tamponade (12). A systematic review and meta-analysis of five studies showed that the application of SEMS achieved hemostasis in 93.5% of cases, whereas rebleeding was present in 13.2% of cases (40).

Transjugular intrahepatic portosystemic shunt (TIPS)

TIPS is presented as intrahepatic (endovascular) shunt procedure. TIPS is recommended for refractory variceal hemorrhage and a better option is the new version of polytetrafluorethylene covered TIPS (PTFE-covered TIPS). Rescue TIPS within 72 h, ideally within 24 h, of variceal bleeding is a good option if there are possibilities for it, i.e. if the bleeding is from EV or from type 1 and 2 GV, as well as if the patient is in Child-Pugh C class of liver cirrhosis with less of 14 points, Child-Pugh B class with over 7 points or with HVPG over 20 mmHg at the time of bleeding. TIPS would be ineffective if the patient is in Child-Pugh C cirrhosis with over 14 points, MELD

score over 30, and lactate over 12 mmol/L, even if liver transplantation is certain in the short term. The decision to use TIPS in these patients is case-specific (11).

A retrospective study by Maimore et al. (41) on 144 patients, with a mean MELD score of 18.5 ± 8.3, of which 8% were in Child-Pugh A class, 38% in Child-Pugh B class and 54% in Child-Pugh C class of cirrhosis, showed failure of TIPS treatment in 16% of cases. Six-week and 12-month mortality was 36% and 42%, respectively. Salvage TIPS was futile in patients with a Child-Pugh score of 14-15.

Surgical treatment

Emergency surgery has limited options in acute variceal bleeding and can be considered as rescue therapy in case of failure of all non-surgical methods including TIPS. Surgery may also be an option in refractory variceal hemorrhage in centers that do not apply radiological interventional procedures (1).

Surgical methods include non-shunt and shunt operations

Non-shunt operations include esophagogastric devascularization, esophageal transection, and splenectomy (Sugiura procedure). These surgeries are rarely the treatment of choice in acute variceal bleeding, but may be salvage therapy when non-surgical and radiologic procedures fail. Also, in cases where it is not possible to perform shunt operations due to extensive portal, splenic, and mesenteric venous thrombosis, devascularization procedures should be considered. Operative mortality is high, especially in patients in the Child C stage of liver cirrhosis, and the Child C stage is also a relative contraindication for this procedure (42). Recent trials have focused on the comparative analysis of laparoscopic and open splenectomy and esophagogastric devascularization. Deng et al. (43) in a retrospective study found no significant difference in hospital mortality due to variceal bleeding between laparoscopic and open surgery. However, with open surgery, there was more intraoperative blood loss, longer hospitalization, and a higher rate of postoperative complications. Luo et al. (44) in a retrospective study that included 30 patients who underwent laparoscopic surgery and 38 patients who underwent open surgery, showed that in both groups of patients there was a significant improvement of varices, evaluated endoscopically. In the laparoscopic group, there was a shorter operative time, less intraoperative bleeding and fewer postoperative complications. In both groups of patients, there was no rebleeding from the varices and no death one year after surgical treatment.

Surgical shunt operations (extrahepatic shunts) are indicated in patients in Child A class with recurrent variceal bleeding despite the application of all non-surgical methods. Decompressive shunts include total portosystemic shunt, partial portosystemic shunt, and other selective shunts.

To create a lateral-lateral total portocaval shunt, the portal vein and the inferior vena cava are mobilized after dissection and anastomosed. All portal flow is directed through the shunt, which is over 10 mm in diameter, with the portal vein itself serving to drain obstructed hepatic sinusoids. With this intervention, good control of variceal bleeding and ascites is achieved in over 90% of cases. Encephalopathy and progressive liver failure are possible in 40-50% of cases.

The partial portosystemic shunt is with a reduced size of the lateral lateral shunt anastomosis to 8 mm. Portal pressure is reduced to 12 mm Hg and portal flow is maintained in 80% of cases. Prospective randomized controlled studies have shown the control of variceal bleeding in 90% of cases, while maintenance of portal flow reduces the incidence of encephalopathy and liver failure.

Selective shunts enable selective decompression of varices with the aim of controlling bleeding and at the same time maintaining portal hypertension while preserving portal flow of the liver. The most commonly used shunt of this type in refractory variceal hemorrhage and in patients with good liver function is the distal splenorenal shunt (Warren shunt). With this shunt, varix decompression is achieved through the short gastric veins and the splenic vein to the left renal vein. In this way, long-term maintenance of portal flow and liver function is ensured with a significantly lower incidence of encephalopathy (10-15%) compared to total portosystemic shunts (45).

TREATMENT OF ACUTE BLEEDING FROM GV

The initial treatment of bleeding from GV does not differ from the treatment of bleeding from EV (restrictive approach to blood transfusions, vasoactive

drugs, antibiotic prophylaxis). Bleeding from GV is less frequent than bleeding from EV but is significantly more severe with higher mortality and more frequent treatment failure (46).

Initial hemostasis in bleeding from GOV1 is achieved with approximately equal efficacy with cyanoacrylate (CYA) tissue adhesive and EBL. In terms of rebleeding from GOV1, CYA has an advantage over EBL. In GOV2 and IGV, the therapy of choice is CYA, which is in the standard form of N-butyl-2-cyanoacrylate. A better alternative is 2-octyl cyanoacrylate, which has a longer polymerization period (11).

CYA injections can also be administered under endoscopic ultrasound (EUS) control. According to many studies, EUS-guided injection of CYA allows for a smaller volume of given CYA, which may affect lower number of complications and less involvement of variceal rebleeding (47, 48).

Recently, the use of EUS-guided coil injections with or without CYA has begun. Coil enables primary hemostasis, keeping CYA inside the varix, thus reducing the risk of embolization. According to a larger retrospective study by Bhat et al. (49), which analyzed 152 patients over a six-year period, it was shown that combined therapy with EUS-guided coil injections and CYA, in high-risk fundic varices, is highly effective in the hemostasis of active bleeding both in primary and secondary bleeding prophylaxis. After the obliteration of the varix was achieved during a longer follow-up period, rebleeding occurred in only 3% of cases. The conclusion of this study is that combination therapy appears to be safe and may reduce the risk of embolization with CYA.

In case of failure of endoscopic hemostasis and early recurrent bleeding from GV according to ESGE recommendations, rescue therapy would be TIPS and balloon-occluded retrograde transvenous embolization (BRTO). Comparing TIPS and BRTO, it can be said that TIPS is associated with a higher risk of encephalopathy, while BRTO is associated with EV deterioration over a longer period of time. Patient selection is important, but due to insufficient comparative data, specific selection criteria are lacking (12).

One randomized controlled trial showed that portocaval shunt surgery demonstrated better control of variceal bleeding, longer survival, and less involvement of encephalopathy compared with emergency TIPS procedure (50). However, further studies are needed before making a decision on the use of portocaval shunt surgery as a salvage procedure after failure of initial treatment of variceal bleeding.

SECONDARY PROPHYLAXIS OF VARICEAL BLEEDING

The indication for the use of secondary prophylaxis of variceal bleeding is the prevention of rebleeding, which occurs in 60% of cases in the first year with a mortality rate of 33%. After repair of acute variceal bleeding, the recommendation of ESGE is that in order to prevent secondary bleeding, NSBB (propranolol) or carvedilol should be used in combination with EBL (strict recommendation, high level of evidence) (12). This position is based on several meta-analyses, according to which combined therapy with NSBB and EBL is superior to monotherapy in EV bleeding (51–53). In the case of recurrent ascites, the treatment of choice in the secondary prophylaxis of variceal bleeding is the application of TIPS. The benefit of TIPS should be evaluated even without the presence of recurrent ascites in case of intolerance or lack of response to NSBB (11).

Regarding the secondary prophylaxis of bleeding from cardiofundal varices (GOV2, IGV1), there is a lack of well-documented data that would be based on evidence from larger studies, and the approach is individual and includes the use of endoscopic CYA injections with or without NSBB, EUS-guided coil injections and CYA, TIPS, and BRTO (12).

CONCLUSION

The most common and severe cause of GI bleeding in patients with portal hypertension is ruptured esophagogastric varices. In patients with advanced liver disease and endoscopically diagnosed high-risk varices, primary prophylaxis of bleeding from varices is carried out using NSBB (propranolol) or carvedilol, while in case of intolerance to NSBB or contraindications to their use, EBL should be used. NSBB or EBL significantly reduce the risk of a first episode of variceal bleeding. In the primary prophylaxis of GV bleeding, in patients with Sarin GOV2 and IGV1, who do not tolerate NSBB, the option of only monitoring without treatment, CYA injections or EUS-guided coil therapy with CYA in centers with experience in the application of this technique is possible. Treatment of acute variceal bleeding involves hemodynamic re-

suscitation of the patient, antibiotic prophylaxis and the earliest possible application of vasoactive drugs. Endoscopy after hemodynamic stabilization would ideally be performed within 12 hours of the presentation of bleeding and EBL applied. Further studies of the role of hemostatic spray/powder in the treatment of acute and refractory variceal hemorrhage are needed. In case of failure of the initial endoscopic treatment, repeat endoscopy and hemostasis should be attempted, followed by balloon tamponade, SEMS, TIPS, and surgical therapy. SEMS may be a better option than balloon tamponade, but larger studies are needed to evaluate the cost-effectiveness of SEMS. Rescue TIPS within 72 h, ideally within 24 h, of variceal bleeding is a good option if the bleeding is from EV or from type 1 and 2 GV, as well as if the patient is in Child-Pugh C class of liver cirrhosis with less than 14 points, Child-Pugh B class with over 7 points or with HVPG over 20 mmHg at the time of bleeding. Emergency surgery (shunt and non-shunt operations) has limited possibilities in acute variceal bleeding and can be considered as rescue therapy in case of failure of all non-surgical methods including TIPS. Surgery may also be an option in refractory variceal hemorrhage in centers that do not apply radiological interventional procedures. In GOV2 and IGV, the therapy of choice is CYA. Further trials of EUS-guided coil injections with or without CYA in the treatment of GV bleeding are needed. The latest recommendation for secondary prophylaxis of EV bleeding is combined therapy with NSBB or carvedilol and EBL. In case of recurrent ascites, the therapy of choice is TIPS. In the secondary prophylaxis of bleeding from cardiofundal varices (GOV2, IGV1), the approach is currently individual due to the lack of more valid evidence from larger studies on the type of therapy.

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Najnovije preporuke u profilaksi i lečenju krvarenja iz ezofagogastričnih variksa

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SAŽETAK

Uvod. Ezofagogastrični variksi razvijaju se kod 50%–60% bolesnika sa cirozom jetre, a 30% njih ima jednu epizodu hemoragije variksa u periodu od dve godine nakon postavljanja dijagnoze variksa. Cilj rada bio je da prikaže najnovije stavove u tretmanu ezofagogastričnih variksa.
Pregled literature. Prevencija prvog krvarenja iz ezofagijalnih variksa (EV) podrazumeva primenu neselektivnih beta-blokatora (engl. *non-selective beta-blockers* – NSBB) ili karvedilola. U slučaju netolerancije ili kontraindikacija prilikom primene treba uraditi endoskopsko ligiranje prstenovima (engl. *endoscopic band ligation* – EBL). Prilikom akutnog krvarenja iz variksa treba uraditi endoskopiju, najpogodnije unutar 12 sati od prezentacije krvarenja, i primeniti EBL. U slučaju refraktarne hemoragije (oko 20%) potrebni su ponovna endoskopija i hemostaza ili tamponada balonom, samoekspandirajući metalni stent (engl. *self-expandable metalic stent* – SEMS), TIPS (engl. *transjugular intrahepatic portosystemic shunt* – TIPS) i hirurška terapija. Krvarenje iz gastričnih variska (GV) ređe je od krvarenja iz EV-a, ali je i znatno teže, sa višim mortalitetom i češćim neuspelim tretmanima. Terapija izbora jeste primena cijanoakrilata (engl. *cyanoaciylates* – CYA), koji se može aplikovati pod kontrolom endoskopske ultrasonografije (engl. *endoscopic ultrasound* – EUS). U ispitivanju su korišćene injekcije kalemovima sa cijanoakrilatom ili bez cijanoakrilata. Kod sekundarne profilakse krvarenja iz EV-a treba primeniti NSBB u kombinaciji sa EBL-om. Kod sekundarne profilakse krvarenja iz kardiofundalnih variksa pristup je individualan.
Zaključak. Terapiju izbora kod primarne prevencije krvarenja iz EV-a predstavlja NSBB, dok je kombinovana terapija (NSBB i EBL) terapija izbora kod sekundarne profilakse krvarenja. CYA je terapija izbora kod krvarenja iz GV-a. Refraktarna variksna hemoragija zahteva primenu mnogih terapijskih modaliteta.

Ključne reči: **ezofagogastrični variksi, profilaksa, tretman**

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Original article

Targeting Inflammation: Cohort Study of the Influence of Methotrexate Therapy on Sideropenic Anemia and Reduction of Inflammatory Markers in Rheumatoid Arthritis Patients

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SUMMARY

Background/Aim. Rheumatoid arthritis (RA) is a systemic autoimmune disease that can cause destructive joint disease and progressive disability. The diagnosis of RA is based on laboratory and clinical evidence, which includes the analysis of inflammatory markers, hematological, and biochemical parameters.
Methods. Fifty patients diagnosed with RA without methotrexate (MTX) therapy and 50 patients with therapy (MTX, 7.5 mg/week; after three months prednisolone 10 mg/day) were included in this study. After six months of therapy, inflammatory biomarkers, hematological, and biochemical parameters were analyzed.
Results. Inflammatory biomarkers: sedimentation rate (SE), C-reactive protein (CRP), and anti-cyclic citrullinated peptide (anti-CCP) are significantly lower in the group of patients on therapy compared to patients without MTX therapy. Significant differences were not found for the rheumatoid factor (RF). Significant differences were not found for hematological parameters between the compared groups. Analysis of serum biochemical parameters showed significant differences for aspartate aminotransferase (AST) and iron values. In patients without MTX therapy, the incidence of anemia was recorded in 68%, which is significantly higher than the incidence of 32% in patients with therapy.
Conclusion. Prescribed therapy has shown effectiveness in the treatment of RA and reduction of the inflammatory process. The success of the treatment depends on the timely diagnosis of RA. Postponement of therapy and late-detected disease prolongs therapy treatment and often requires a combination of several drugs.

Keywords: **rheumatoid arthritis, anti-cyclic citrullinated peptide, C-reactive protein, methotrexate**

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic and progressive autoimmune disease, which causes joint impairment, arthralgia, joint swelling, disability, and shortened life expectancy (1). It is characterized by erosive synovitis associated with many inflammatory conditions (2). Patients diagnosed with RA have an increased risk of coronary heart attack, arterial sclerosis, and stroke (3). The diagnosis of RA is based on laboratory and clinical evidence that includes determination of inflammatory markers: rheumatoid factor (RF) test, erythrocyte sedimentation rate (SE), C-reactive protein (CRP), and cyclic citrullated peptide antibody test **(**anti-CCP test) and a blood count. The origin of RA is not fully understood, but current research suggests a combination of several possible factors, such as an abnormal autoimmune response, genetic predisposition and viral and/or bacterial infection.

Common symptoms of RA include morning stiffness, fatigue, fever, weight loss, tenders, swollen and warm tenders, and rheumatoid nodules under the skin. The onset of this disease is usually from the age of 35 to 60 years, with remission and exacerbation (4, 5). The aim of treating rheumatoid arthritis is clinical remission of the disease. If the treatment of RA is prolonged, progressive bone impairment occurs, along with the appearance of joint deformities with a complete loss of their function (6, 7). Treatment for RA is used to reduce joint inflammation and pain, maximize joint function, and prevent joint destruction and deformity. Treatment regimens consist of combinations of pharmaceuticals, weight-bearing exercise, educating patients about the disease and rest. Treatments are generally customized to a patient's needs and depend on their overall health. This includes factors such as disease progression, age, overall health, occupation, compliance and education about the disease.

Treatment of RA includes the use of glucocorticosteroids (GCs), most often in combination with other antirheumatic drugs–disease modifying antirheumatic drugs (DMARD) (8-10). A large number of GCs are available whose main role is to reduce inflammatory processes and pain, and their use depends on the degree of tissue involvement and the duration of the disease. Well-known drugs from the group of GCs are: budenofalk, dexamethasone, prednisolone, and berlicort. DMARDs are recommended in the first three months after the onset of rheumatoid arthritis symptoms with the aim of reducing inflammatory processes, improving joint function, achieving remission, and preventing permanent damage (11-13).

Well-known drugs from this group are: methotrexate (MTX), leflunomide, antimalarial, penicillamine, hydroxychloroquine, and sulfasalazine (14). Treatment of RA begins with MTX, which has been declared the essential drug by the World Health Organization (WHO). MTX is a structural analogue of folic acid that competitively inhibits the binding of dihydrofolic acid (FH2) to the enzyme that is responsible for converting FH2 to folinic acid (FH4). Without FH4, the metabolism of purine and pyrimidine is impaired, and the synthesis of amino acids and polyamine is inhibited (15). MTX prevents further permanent damage that occurs if rheumatoid arthritis is left untreated (3). The dosage of MTX depends on the activity, duration of the disease, age and comorbidities; standard doses are from 7.5 to 25 mg per week. Side effects caused by MTX can be symptomatic (nausea, headache, fatigue, mucositis) and potentially life-threatening (cytopenia, hepatotoxicity, pulmonary damage, sudden vision loss, and nephrotoxicity) (16).

The goal of our research was to analyze the effectiveness of RA patient therapy at the hematological, biochemical, and inflammatory levels.

PATIENTS AND METHODS

Participants and inclusion criteria

This study included 100 patients diagnosed with rheumatoid arthritis (RA). Clinical parameters for patients in these studies were taken from the database of the University Clinical Hospital in Mostar (Bosnia and Herzegovina). Access and use of data was approved by the Ethics Committee of the University Clinical Hospital Mostar, number: 834/21. Diagnosed RA and informed consent of all participants were the criteria for inclusion in the study. The diagnosis of RA was made based on the patient's history, physical examination, laboratory and radiological findings. The subjects were divided into two groups of 50 patients each, patients on therapy (Th), and patients without MTX therapy (NTh). Therapy included methotrexate (MTX) in the initial stage of the disease, 7.5 mg per week, according Lopez-Olivo MA et al. (17) weekly doses that ranged between 5 mg and 25 mg, and after three months prednisolone

10 mg (1 x 1). After six months on therapy, blood was sampled for analysis. The study included patients who were not on therapy, and based on the anamnesis, it was determined that the disease had been present for an average of six months. Patients without MTX therapy were under medical supervision and used non-steroidal anti-inflammatory drugs (NSAIDs), and according to ACR/EULAR criteria and DAS28-ESR score, did not require treatment with the use of DMARDs (18).

Inflammatory markers

The analysis of inflammatory markers included the titer of rheumatoid factor (RF), the value of anti-citrulline antibodies (anti-CCP), erythrocyte sedimentation rate (SE), and the value of C-reactive protein (CRP). The immunoturbidimetric method was used to analyze RF and CRP; the parameters were analyzed using a Beckman Coulter DxC 700 AU analyzer. SE value was determined by photometric principle using iSED®, fully-automated ESR analyzer, while anti-CCP was analyzed by ELISA method.

Biochemical parameters

Analysis of biochemical parameters included values of urea, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), glucose, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), total cholesterol, triglycerides, and iron (Fe). All biochemical parameters were analyzed on a Beckman Coulter DxC 700 analyzer. Glucose was determined by the hexokinase method. HDL, LDL and triglycerides were determined by an enzymatic staining test. The photometric UV method was used to analyze enzyme activity (AST, ALT, and GGT). Iron was determined by a photometric staining test.

Hematological parameters

The analysis of hematological parameters included the number of platelets, erythrocytes and leukocytes, hemoglobin concentration, hematocrit values, values of the hematological indices: mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The mentioned hematological parameters were analyzed using an automated hematological analyzer Sysmex XN 1000 (Sysmex Corporation, Kobe, Japan).

Statistical analysis

Statistical analysis data was performed by variance analysis (ANOVA) using the IBM SPSS (Version 20.0, SPSS, Inc., Chicago, IL, USA). Differences between the groups were determined by a range test ($p < 0.05$ and $p < 0.001$). The Pearson's correlation coefficient was used as a measure of the strength of the linear association between the two variables.

RESULTS

Figure 1 presents the analysis of inflammatory markers. By comparing the results, significant values for CRP (Th = 12.15 mg/L; NTh = 23.12 mg/L), SE (Th = 20.98 mm/h; NTh = 40.60 mm/h) and anti-CCP (Th = 215.35 IU/mL; NTh = 355.07 IU/mL) were determined. Rheumatic factor values (Th = 89.26 IU/mL; NTh = 107.06 IU/mL) did not differ between the compared groups. The greatest variations of inflammatory markers were recorded for SE values. High individual variations were recorded in both examined groups.

Analysis of hematological parameters (Table 1) did not reveal statistically significant differences between the compared groups. Higher values of PLT and MCHC were recorded in the group of patients without therapy, while other observed parameters had similar average values.

The values of biochemical parameters are shown in Table 2. Statistically significantly higher values were recorded for the concentration of Fe and AST in Th group.

Table 3 shows the assessment of genetic predisposition for the development of RA. In 18% of respondents from the Th group, there is a genetic predisposition to the rheumatoid arthritis occurrence. In the NTh group, genetic predisposition was found in 28% patients. Although a genetic predisposition was confirmed, it was not significant.

The presence of anemia was recorded in 32% of subjects from the Th group and in 68% of patients from the NTh group (Table 4). Anemia of chronic disease was present in 15% of patients in the Th group, in 85% in the NTh group; 55% of subjects have sideropenic anemia in the Th group, and 45%

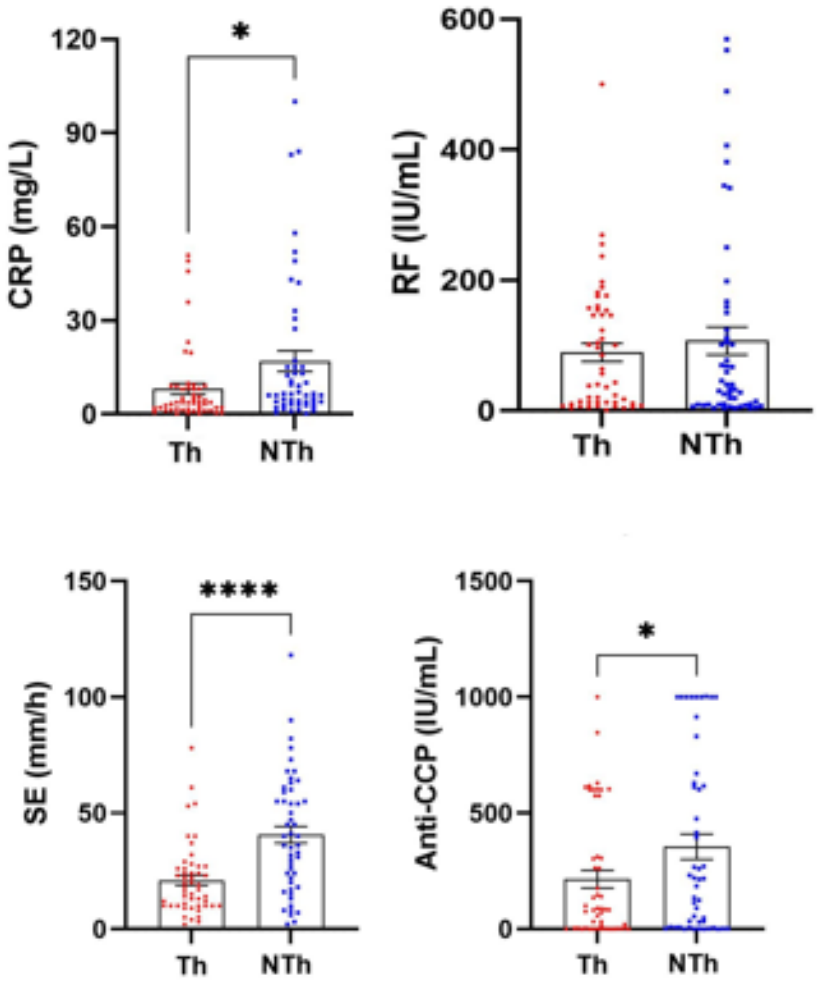

Figure 1. *Inflammatory biomarker values between groups (CRP—C reactive protein, SE— sedimentation rate; anti-CCP antibody; RF—rheumatoid factor). Th—patients with MTX therapy; NTh—patients without MTX therapy. *Significant at 0.05; ****Significant at 0.001. Data are presented as average and median*

Table 1. *Overview of hematological parameters*

Parameters	Reference range	Th	NTh	Sig.
RBC (x10^{12}/L)	4.20–5.90	4.56 ± 0.32	4.49 ± 0.48	0.371
WBC (x10^{9}/L)	4–10	8.01 ± 2.20	8.05 ± 2.37	0.920
PLT (x10^{9}/L)	150–400	252.32 ± 65.62	291.08 ± 78.31	0.270
Hb (g/L)	120–170	132± 12.95	131.10 ± 15.05	0.525
HCT (L/L)	0.356–0.510	0.40 ± 0.03	0.39 ± 0.04	0.203
MCV (fl)	83.0–97.2	88.03 ± 5.66	86.97 ± 4.78	0.313
MCH (pg)	27.4–33.9	29.07 ± 2.55	29.06 ± 2.31	0.977
MCHC (g/L)	320–345	331.24 ± 12.43	335.50 ± 14.07	0.111

Table 2. *Overview of biochemical parameters*

Parameters	Reference range	Th	NTh	Sig.
Fe (μmol/L)	8–30	15.30 ± 5.47	11.52 ± 4.28	0.000**
Urea (mmol/L)	3.0–9.2	5.71 ± 1.57	5.53 ± 1.97	0.620
Creatinine (μmol /L)	63.6–110.5	68.29 ± 15.52	73.94 ± 14.37	0.062
AST (U/L)	8–38	21.84 ± 6.09	18.75 ± 4.90	0.006*
ALT (U/L)	10–48	24.05 ± 11.27	20.31 ± 9.07	0.071
GGT (U/L)	10–50	20.91 ± 13.41	21.11 ± 12.87	0.939
GUK (mmol/L)	4.4–6.4	5.54 ± 1.34	6.24 ± 2.58	0.088
HDL (mmol/L)	1.03–1.55	1.78 ± 0.49	1.77 ± 0.57	0.872
LDL (mmol/L)	1.55–4.53	3.32 ± 0.77	3.45 ± 0.96	0.457
HOL (mmol/L)	3.1–5.5	5.74 ± 0.95	5.67 ± 1.09	0.756
TRIG (mmol/L)	0.46–2.28	1.49 ± 0.57	1.52 ± 0.62	0.770

*Significantly different at 0.05 and ** at 0.001

Table 3. *Genetic predisposition to the rheumatoid arthritis occurrence*

			Th	NTh	Total
Genetic predisposition	NO	N	41	36	77
		%	82	72	
	YES	N	9	14	23
		%	18	28	
Total		N	50	50	100
		%	100	100	
Pearson Chi-Square		F = 1.412 (p = 0.235)			

Table 4. *Types of anemia within the examined groups*

		Anemia presence		Anemia type	
		YES	NO	Anemia of chronic disease	Sideropenic anemia
Th	N	7	43	2	5
	%	32	55	15%	55%
NTh	N	15	35	11	4
	%	68	45	85%	45%
Pearson Chi-Square				F = 3.730 (p = 0.053)	

Table 5. *Correlation relationship of SE. RF and anti-CCP within the examined groups*

		SE-RF	SE-Anti-CCP	RF-Anti CCP
T-test	Th	0.323	0.000**	0.019
	NTh	0.649	0.007*	0.931

*Significantly different at 0.05 (P<0.05) and **at 0.00 (p < 0.001)

in the NTh group. Confirmed anemia was not significant when comparing both groups.

By comparing the values of inflammatory markers within the Th group (Table 5), a statistically significant correlation was found between the values of SE and anti-CCP, as well as RF and anti-CCP, while statistically significant correlation was not observed between the values of SE and RF. A statistically significant correlation in the NTh group was observed only between SE and anti-CCP values.

DISCUSSION

Rheumatoid arthritis (RA) is a chronic, inflammatory, systemic autoimmune disease affecting the joints with varying severity among patients. The risk factors include age, gender, genetics, and environmental exposure (8). As there is no cure for RA, the treatment goals are to reduce the pain and stop/slow further damage (19). Inflammatory markers (SE, RF, anti-CCP, and CRP) have great diagnostic significance in patients with RA. In our study, the six-month treatment of patients diagnosed with RA showed a statistically significant decrease in inflammatory markers (SE, CRP, and anti-CCP) in the Th group compared to the NTh group; significant differences were not found for the rheumatism factor RF. The research conducted by Shrivastava et al. (20) on 110 RA patients showed statistically significantly higher values of inflammatory markers compared to the control group. Patients with RA have high levels of inflammatory markers, and that it is very important to start treatment in a timely manner. Ruof et al. (21) conducted research on 200 RA patients and found a significant correlation between the inflammatory markers SE and CRP. The obtained results support the point of view on the simultaneous use of SE and CRP and their significance in the clinical practice of rheumatologists. In the research of Alessandri et al. (22), the effect of infliximab treatment on inflammatory markers anti-CCP and RF in patients with rheumatoid arthritis was investigated. At baseline, 38 of 43 patients (88%) were positive for anti-CCP antibodies and 41 (95%) were positive for RF. The values of inflammatory markers anti-CCP and RF significantly decreased after six months of treatment, which suggests that these measurements may have diagnostic significance in evaluating the effectiveness of RA treatment. The conclusions of this study are in correlation with current results because statistically significantly lower values of the inflammatory markers RF and anti-CCP were found in the Th group compared to the values of the inflammatory markers of patients from the NTh group.

Most rheumatologists believe that MTX has a major role in the treatment of RA, alone or in combination with other DMARDs. Because MTX had a dominant therapeutic role, the new drugs were also studied in combination with it. Cohen et al. (23) investigated the effectiveness of combined therapy in 419 RA patients for six months. The combination of MTX with another drug (anakinra) was safe and well tolerated and provided significantly greater clinical benefits than MTX alone. Maini et al. (24) demonstrated in a two-year study that infliximab and MTX provided significant, clinically relevant improvement in physical function and quality of life, accompanied by inhibition of progressive joint impairment and sustained improvement in the signs and symptoms of RA among patients who had previously incomplete response to MTX alone. Visser and der Heijde (25) concluded that any new antirheumatic drug must be combined with MTX. RA treatment is continuous and lasts as long as the drug is effective or until side effects appear. The most common hematological side effect in the treatment of RA with high doses of MTX is myelosuppression. Changes in hematological parameters caused by treatment with MTX can lead to macrocytosis, however, pancytopenia is not excluded either (26). MTX is excluded in patients whose MCV value is higher than 110 fL, i.e. if there is significant macrocytosis (27). Thrombocytopenia, leukopenia, and anemia are successfully regulated by lowering the dose and stopping taking the medication, depending on the intensity of the disorder. During treatment with MTX, monthly controls of liver and kidney function and complete blood counts are required (28). The values of hematological parameters are not statistically significant when comparing the two examined groups in the present study. A study of Dechanuwong and Phuan-Udom (29), including 365 patients with RA, proved that there are changes in the hematological profile of patients that are related to the activity of the disease. The findings of this study show that the level of hemoglobin (Hb), neutrophil/lymphocyte ratio (NLR), and mean platelet volume (MPV) are independent factors for disease activity in RA. Numerous studies indicate that hematological parameters such as leukocytes (WBC), hemoglobin (Hb), and platelets are associated with inflammatory pro-

cesses in patients with RA (30-32). Some authors found lower values of Hb and mean platelet volume (MPV) (33-35), but higher neutrophil/lymphocyte ratios (NLR) and platelet count (36) in patients with RA.

Statistically significantly higher values of the examined biochemical parameters were recorded for iron concentration and AST in patients on therapy (Th) in the current study. Kremer et al. (37) investigated the effectiveness of MTX therapy on AST and ALT values in patients suffering from RA. The research results showed a statistically significant decrease in AST and ALT values after a period of six months. Biochemical markers of bone and cartilage turnover are also receiving increasing attention in other conditions characterized by joint and/or skeletal inflammation and damage. They may provide an additional and potentially more sensitive method of detection of active bone and cartilage degradation that is likely to lead to structural damage in RA (38).

Pallinti et al. (39) found that ferritin levels are not significant in RA patients. Smith et al. (40) analyzed 35 anemic patients with rheumatoid arthritis to determine the relationship between serum iron levels and its body status by assessing bone marrow iron stores. The study shows that reduced bone marrow iron stores are common in patients with rheumatoid arthritis, and that serum ferritin levels may be a useful indicator of reduced body iron stores in these patients. In the present study, the incidence of anemia in patients from the NTh group was recorded in 68%, which is significantly higher than the incidence of 32% in patients from the Th group. Anemia of chronic disease and sideropenic anemia are the two most common types of anemia in RA patients. Anemia of chronic disease in Th group was found in 15% of patients, and in NTh group was recorded in 85% of the total number of 13. Sideropenic anemia in group Th was found in 55% of patients, and in the NTh group in 45% of patients. Research of Peeters et al. (41) included 225 patients with RA for two years; anemia was recorded in 64% of patients, of which 77% had anemia of chronic disease, and 23% of patients had anemia due to iron deficiency. Our research was in correlation with the previeous study, considering that among NTh group patients, anemia of chronic disease was the most prevalent (85%), and more than half of NTh group patients were anemic (68%).

CONCLUSION

MTX in combination with prednisolone is an effective therapy for the treatment of RA, however, therapy did not reduce the high values of the RF. Significant variations were not found for hematological parameters, but biochemical parameters showed a significant decrease in AST activity and iron. It seems that the analysis of inflammatory markers with serum iron could be very important for monitoring the therapy of RA patients due to the high incidence of anemia of chronic disease and sideropenic anemia associated with inflammatory changes in rheumatoid arthritis. Further studies are needed to allow efficacious and cost-effective drugs to be used to prevent the long-term complications of uncontrolled RA.

Authors' contribution

Study conception and design: DS, EM, MF. Data collection: MMB. EM. Data analysis: DS. Data interpretation: EM, DS. Drafting the manuscript: MMB, DS. Revising the manuscript critically for important intellectual content: MM, DS, MF. All authors approved the final version of the manuscript.

Declarations

Conflict of interest

The authors declare no competing interests.

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Fokus na inflamaciju: kohortna studija o uticaju terapije metotreksatom na sideropenijsku anemiju i smanjenje inflamatornih markera kod bolesnika sa reumatoidnim artritisom

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SAŽETAK

Uvod/Cilj. Reumatoidni artritis (RA) jeste sistemska autoimuna bolest koja može uzrokovati degenerativno oboljenje zglobova i progresivni invaliditet. Dijagnostika RA zasniva se na laboratorijskim i kliničkim dokazima koji obuhvataju analizu upalnih markera, hematoloških i biohemijskih parametara.
Metode. U studiju je uključeno 50 bolesnika sa dijagnozom RA koji nisu lečeni metotreksatom (engl. *methotrexate* – MTX) i 50 bolesnika koji jesu lečeni metotreksatom (7,5 mg/nedeljno), a nakon tri meseca prednizolonom (10 mg dnevno). Nakon šestomesečne terapije, analizirani su inflamatorni biomarkeri, hematološki i biohemijski parametri.
Rezultati. Inflamatorni biomarkeri (sedimentacija eritrocita – SE; C-reaktivni protein – CRP i antitela na ciklični citrulinski peptid (engl. *anti-cyclic citrullinated peptide* – anti-CCP)) signifikantno su niži u grupi bolesnika koji su primali terapiju nego u grupi bolesnika koji nisu bili na terapiji MTX-om. Nisu utvrđene značajne razlike za reumatoidni faktor (RF). Između poređenih grupa nisu utvrđene signifikantne razlike za hematološke parametre. Analiza serumskih biohemijskih parametara pokazala je signifikantne razlike za aspartat aminotransferaze (AST) i gvožđe. Kod bolesnika koji nisu primali terapiju MTX-om incidencija anemije zabeležena je kod 68%, što je značajno više u odnosu na incidenciju od 32% kod bolesnika koji jesu primali terapiju.
Zaključak. Ordinirana terapija pokazala je efikasnost u lečenju RA i u redukciji inflamatornog procesa. Uspešnost lečenja zavisi od pravovremene dijagnoze RA; odlaganje terapije i kasno detektovana bolest produžavaju terapiju koja često zahteva kombinaciju više lekova.

Ključne reči: **reumatoidni artritis, antitela na ciklični citrulinski peptid, C-reaktivni protein, metotreksat**

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Original article

Evaluation of the Mandibular Canal Course in Southeast Serbian Population: A Cone Beam Computed Tomography Study

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SUMMARY

Introduction/Aim. Oral surgical interventions in the mandibular region require detailed knowledge of the position and course of the mandibular canal. The aim of this study was to determine the most common course of mandibular canal on cone beam computed tomography (CBCT) images in the population of Southeast Serbia.

Material and method. One hundred ninety-four mandibular canals on CBCT images of 97 patients (48 male and 49 female) aged 18-65 years were analyzed in the study. According to Worthington, courses of mandibular canals are classified into catenary, descending, and straight. The obtained results were analyzed in relation to the gender and the age of the patients as well as to the left and right side of the mandible.

Results. The most common course of mandibular canal on the analyzed images was catenary (41.2%), then straight (37.1%), while the least was descending (21.6%). The most common type in males was the catenary (46.9%), while the straight type was the most common in females (39.6%). Statistical analysis showed no significant difference in the distribution of the mandibular canal course in relation to the gender, age of the patients, and the side of the mandible.

Conclusion. The observed variations emphasize the importance of careful individual preoperative analysis of CBCT images of each patient as well as planning different treatment modalities in the region of the mandible.

Keywords: **mandible, mandibular canal, anatomical variation, cone beam computed tomography**

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INTRODUCTION

Oral surgical interventions require detailed knowledge of anatomical structures in the orofacial region. The mandibular canal is a very important anatomical detail of the mandible, due to the importance of the neuro-vascular elements that pass through it. On the inner side of the ramus of the mandible is the initial opening of this canal—the mandibular foramen, into which the inferior alveolar nerve, artery, and vein enter. This canal extends together with inferior alveolar neurovascular bundle along the entire body of the mandible towards the mental foramen, while its branch continues into the contralateral side (1).

Knowledge of variations in the position and course of this canal is of great clinical importance in the prevention of complications caused by iatrogenic injuries of the canal and its contents during surgical teeth extractions (2). Also, precise knowledge and identification of the exact localization of the mandibular canal is extremely important in planning the position of dental implants, which are becoming an increasingly common choice for lost teeth replacing (3). Surgical removal of periapical lesions, which are located on the roots of the teeth in the immediate vicinity of the mandibular canal, in some cases described in the literature, is accompanied by postoperative neurosensitive complaints (4).

Cone beam computed tomography (CBCT) is one of the most useful diagnostic procedures in oral surgery because it shows a very precise insight into the localization of the anatomical structures of the examined area (5). Using this method, numerous authors have described the different relationships of the mandibular canal with the roots of premolars and molars, where the most common finding is that the mandibular canal is closest to the second molar (6, 7). The literature review also points out that the mandibular canal is closer to the buccal cortex of the mandible on the right side (7). The variations of the different types of mandibular canal course are precisely described by the classification according to Worthington (8), who stated that the mandibular canal can be classified as catenary, descending, and straight.

The aim of this study was to determine the most common course of the mandibular canal on CBCT images in the population of Southeast Serbia.

MATERIAL AND METHODS

Undertaking of this study was approved by the Ethics Committee of the Clinic of Dental Medicine in Niš (01-728/23). The study analyzed 194 CBCT images of the mandibular canals from 97 adults (48 male and 49 female) aged 18-65 years, who came to the Clinic of Dental Medicine in Niš. The inclusion criteria for examining the images were alveolar processes of the mandible with full dentition where the entire mandibular canals were visible, while edentulous jaws and mandibles with pathological conditions, supernumerary, and impacted teeth were not analyzed. The images were taken with the Galileos Comfort Plus scanner (Sirona Dental Systems, Germany). In order to avoid subjectivity and non-uniformity in classifications, mandibular canals on CBCT images were examined and classified by one experienced oral surgeon. The analysis of the images was performed in the Galileos program (Sirona Dental Systems, Germany). The course of the canal was monitored based on the software marking on the projection of the image in the sagittal plane. In this way, canals were located and classified according to Worthington (8) into catenary, descending, and straight. The catenary canal was in the form of U-like shape, the descending canal was in the form of plunging progressive curve, while the straight canal was steep ascent along with root apexes (Figure 1). The obtained results were analyzed in relation to the gender and the age of the patients, but also in relation to the left and right side of the mandible. The obtained data were analyzed in IBM SPSS version 26.0 using the Chi-square test with a significance of $p < 0.05$.

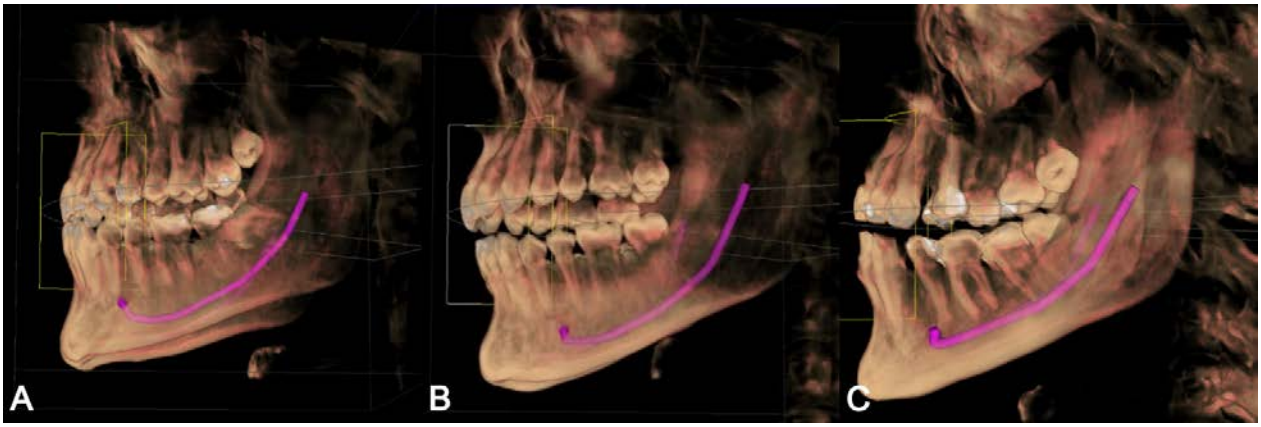

Figure 1. *Different types of mandibular canals' courses (A – catenary, B – descending, C – straight) (Original CBCT)*

RESULTS

The results showed that the most common type of mandibular canal on the analyzed images was catenary (41.2%), then straight (37.1%), while the least present type was descending (21.6%).

The analysis of the results in relation to gender showed that the catenary type was the most common in males with 46.9%, while the most common type in females was straight (39.8%). However, statistical analysis did not show a significant difference in the frequency distribution of mandibular canal course types in relation to gender (Table 1).

The analysis of the results in relation to the age of the patients showed that the catenary type of canal was most often found in patients aged 34-48 and 49-65 years, while in patients aged 18-33 years, the distribution of catenary and straight type was equal. Statistical analysis showed that there was no significant difference in the distribution of canal types in relation to the age of the patients (Table 2).

The analysis of the results in relation to the left and right side of the mandible showed that the most common canal course on the left side was the catenary type (44.3%), while the most common type on the right side was straight (40.2%), however, this difference in the types of courses was not statistically significant (Table 3).

Table 1. *Frequency distribution of mandibular canal types according to gender*

			Canal types			Total	Chi - square
			Catenary	Descending	Straight		
Gender	Male	Count	45	18	33	96	$\chi^2 = 2.587$ df = 2 p = 0.274
		%	46.9%	18.8%	34.4%	100%	
	Female	Count	35	24	39	98	
		%	35.7%	24.5%	39.8%	100%	
Total		Count	80	42	72	194	
		%	41.2%	21.6%	37.1%	100%	

χ^2 – Chi –square test value; df – degree of freedom value, p – the value of the probability of the Chi-square test

Table 2. *Frequency distribution of mandibular canal types according to age group*

			Canal types			Total	Chi - square
			Catenary	Descending	Straight		
Age group	18-33	Count	20	16	20	56	$\chi^2 = 4.220$ df = 4 p = 0.377
		%	35.7%	28.6%	35.7%	100%	
	34-48	Count	27	16	25	68	
		%	39.7%	23.5%	36.8%	100%	
	49-65	Count	33	10	27	70	
		%	47.1%	14.3%	38.6%	100%	
Total		Count	80	42	72	194	
		%	41.2%	21.6%	37.1%	100%	

χ^2 – Chi –square test value; df – degree of freedom value, p – the value of the probability of the Chi-square test

Table 3. *Frequency distribution of mandibular canal types according to anatomic side of mandible*

			Canal types			Total	Chi - square
			Catenary	Descending	Straight		
Anatomic side	Left side	Count	43	21	33	97	$\chi^2 = 0.950$ df = 2 p = 0.622
		%	44.3%	21.6%	34.0%	100%	
	Right side	Count	37	21	39	97	
		%	38.1%	21.6%	40.2%	100%	
Total		Count	80	43	71	194	
		%	41.2%	21.6%	37.1%	100%	

χ^2 – Chi –square test value; df – degree of freedom value, p – the value of the probability of the Chi-square test

DISCUSSION

Although panoramic images are most often used in planning oral surgical interventions, the impossibility of three-dimensional visualization of certain anatomical structures makes the CBCT technology necessary in everyday practice. Due to the growing need for more precise identification of these structures, many researchers have proven that CBCT technology is irreplaceable in determining anatomical and morphological variations (5).

The advantage of this method of imaging is reflected in the fact that patients are exposed to a lower dose of radiation during imaging with CBCT technology compared to imaging using the traditional computerized tomography (CT) method (9). The study of Jung and Cho (10), which examined the visibility of the mandibular canal on X-ray images, concluded that the visibility of the mandibular canal is better on images obtained by CBCT technology than on panoramic images. CBCT technology provides the possibility of viewing the position of the canal in three dimensions, which makes it possible to determine its bucco-oral position (5).

The results of this study showed that the most common course of the mandibular canal in the population of Southeast Serbia was catenary (41.2%). This course was also the most common in the research of Ozturk et al. (11) who determined the direction of the mandibular canal on dry skulls. The studies of Jung and Cho (10) as well as Liu et al. (12), conducted on CBCT and panoramic images, where the canals were classified into four types, showed that the most frequent mandibular canal type was

the one that corresponded to the catenary canal type. The root apexes of premolars and molars are further apart from the mandibular catenary type canal compared to the other types, which greatly reduces complications in form of accidental opening of the canal during the extractions of these teeth. The catenary type of canal is also the most suitable type for placing implants, according to the greatest distance of the mandibular canal from the apex of the lower first molar (13), which, due to frequent extractions, is the tooth that most often needs to be replaced with an implant (14). The importance of this is reflected in the fact that injuries of the mandibular canal are manifested by bleeding or paresthesia and complications that would require additional surgical interventions in order to treat them (2).

The results of this study showed that 37.1% of the respondents had a straight canal type. Data from the literature showed that this type of canal proved to be the most inconvenient during oral surgical interventions, due to the proximity of the premolars and molars to the mandibular canal (13). A similar prevalence of this type of canal was observed in a study by Mirbeigi et al. (15), who examined the mandibular canal course in the Iranian population using the Worthington classification (8). However, in contrast to the results of our study, where the catenary type was the most common, the aforementioned study by Mirbeigi et al. (15) showed that all three types of canals were equally prevalent among patients.

The least represented canal type in this study was the descending type with 21.6%. However, data from the literature showed that this type of canal was dominant in studies in the populations of India and Kenya, where the same methodology was used to classify the course of the mandibular canal on CBCT images (13, 16). Such discrepancies could be explained by population differences, which reflect in different types of mandibular growth (17). Numerous studies highlight such differences between the populations of Europe, India, Israel and South Korea, regarding the different relationships of the posterior teeth with the mandibular canal (18) and the mental foramen (19).

Although the results of this study showed that the catenary type was more common in males, while the straight type was more common in females, statistical analysis showed no significant difference in the frequency distribution of examined types of mandibular canal between gender and the age groups. This result is in agreement with numerous studies that examined the sexual dimorphism of the course of the mandibular canal as well as the frequency of different courses of the mandibular canals in different age groups (13, 15, 20). The analysis of the course in relation to the side of the mandible in this study showed that catenary type was more prevalent on the left side, while the straight one was more common on the right side, however, this difference was not statistically significant. On the contrary, in the study by Viera et al. (20), catenary and descending types were observed significantly more often on the right side.

The limitation of the study is reflected in the impossibility of a wider analysis of patients who had to meet the criteria for participation in the study. Considering that in the population of Serbia, a significant number of people do not have full dentition due to frequent tooth extractions (21), a large number of patients could not participate in the study.

CONCLUSION

Although the catenary type of the mandibular canal was present in the largest number of examined patients within the population of Southeast Serbia, a significant number of patients were identified with the straight type of the mandibular canal, which represents a very challenging situation for oral surgical interventions. This emphasizes the importance of careful individual preoperative analysis of CBCT images of each patient as well as planning of different treatment modalities in the region of the mandible.

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Evaluacija toka mandibularnog kanala u populaciji jugoistočne Srbije: studija kompjuterizovane tomografije konusnog zraka

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SAŽETAK

Uvod. Oralnohirurške intervencije u regiji mandibule zahtevaju detaljno poznavanje položaja i toka mandibularnog kanala. Cilj ovog rada bio je da se odredi najčešći tok mandibularnog kanala na snimcima dobijenim pomoću kompjuterizovane tomografije konusnog zraka (engl. *cone-beam computed tomography* – CBCT) u populaciji jugoistočne Srbije.

Materijal i metode. U studiji su analizirana 194 CBCT snimka mandibularnih kanala 97 pacijenata (48 muškaraca i 49 žena) starih od 18 do 65 godina. Prema Worthingtonu, mandibularni kanali dele se na zakrivljene, poniruće i prave. Dobijeni rezultati su analizirani u odnosu na pol i starost pacijenata, kao i u odnosu na levu i desnu stranu mandibule.

Rezultati. Najčešći tok mandibularnog kanala na analiziranim snimcima bio je zakrivljeni (41,2%), pa pravi (37,1%), dok je najmanje zastupljen bio poniruć kanal (21,6%). Kod muškaraca je najzastupljeniji bio zakrivljeni tip (46,9%), dok je kod žena najzastupljeniji bio pravi tip kanala (39,6%). Statistička analiza nije pokazala značajnu razliku u distribuciji različitih tokova mandibularnog kanala u odnosu na pol i starost pacijenata, kao ni u odnosu na stranu mandibule.

Zaključak. Uočene varijacije naglašavaju značaj pažljive individualne preoperativne analize CBCT snimaka svakog pacijenta, kao i planiranja različitih operativnih pristupa u regiji mandibule.

Ključne reči: **mandibula, mandibularni kanal, anatomske varijacije, kompjuterizovana tomografija konusnog zraka**

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Original article

Refining Risk Stratification in Pulmonary Embolism: Integrating Glomerular Filtration Rate and Simplified Pulmonary Embolism Severity Index as a Potent Predictor of Patient Survival

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SUMMARY

Background/Aim. Patients classified as belonging to simplified pulmonary embolism severity index (sPESI) class 0 are considered to have low-risk pulmonary embolism (PE). Yet, certain laboratory and echocardiographic parameters not accounted for in the sPESI score might suggest a likelihood of worse outcomes in PE cases. This study seeks to determine if the prognostic value of the sPESI score in acute PE can be improved, refined, and optimised by incorporating brain natriuretic peptide (BNP) and troponin I (TnI) levels, echocardiographic parameters, or glomerular filtration rate.

Methods. The study encompassed 1,201 consecutive patients diagnosed with PE, confirmed by multidetector computed tomography (MDCT). Upon admission, each patient underwent an echocardiography exam, and blood samples were taken to measure B-type natriuretic peptide (BNP), troponin I (TnI), creatinine, and other routine laboratory markers.

Results. The in-hospital mortality rate was 11.5%. The patients were categorized into three groups using the three-level sPESI model: sPESI 0, sPESI 1, and sPESI ≥ 2. Statistically significant differences were found among these groups regarding mortality rates, TnI values, BNP levels, estimated glomerular filtration rate (eGFR), and the presence of right ventricular dysfunction (RVD). Cox regression analysis identified eGFR as the most reliable predictor of 30-day all-cause mortality [HR 2.24 (CI 1.264-3.969); p = 0.006] across all sPESI categories. However, incorporating TnI, BNP, or RVD did not improve risk prediction beyond the three-level sPESI model.

Conclusion. Renal dysfunction at the time of admission is closely related to an elevated risk of in-hospital mortality in patients with acute PE. The three-level sPESI score offers a more accurate method for prognostic stratification in these patients.

Keywords: **pulmonary embolism, sPESI score, prognosis**

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INTRODUCTION

Pulmonary embolism (PE), being the most severe manifestation of venous thromboembolism (VTE), has a three-month mortality rate ranging from 8.7% to 17.4%, and, as such, it necessitates the prompt evaluation of the patient in an acute care setting (1, 2). The pulmonary embolism severity index (PESI) is one of the most commonly used and thoroughly validated clinical scoring systems available (3, 4). A recent randomized trial identified low-risk pulmonary embolism (PE) classes (PESI classes I and II) as a potential criterion for outpatient treatment of acute PE (5).

This calculation is complex in the emergency department setting due to the need for multiple clinical variables in the acute management of pulmonary thromboembolism (PTE). The simplified PESI (sPESI) score, which is determined using six equally weighted variables—age, chronic lung disease (such as COPD), cancer history, chronic heart failure (CHF), heart rate (HR), systolic blood pressure (BP), and arterial oxyhaemoglobin saturation below 90%—provides dependable prognostic insights (6, 7). A recent study indicated that the sPESI is as reliable as the imaging and biomarker criteria recommended by the European Society of Cardiology (ESC) for identifying low-risk patients (8). However, the therapeutic approach based on sPESI is still a subject of debate. Additionally, several registries have highlighted an increased the occurrence of pulmonary embolism (PE) or venous thromboembolism in individuals with chronic kidney disease (CKD) (9, 10).

It is important to question the reliability of distinguishing between sPESI scores of 1 and 0 when excluding the possibility of adverse outcomes in patients with acute PE (11). Biochemical markers have been suggested to be used as an alternative method employed for risk stratification. Yet, the occurrence and prognostic significance of acute kidney injury or dysfunction, whether present upon admission or developing during hospitalization, have been neglected and underestimated in patients with acute PE (12). In acute situations, alternations and disruptions in pulmonary circulation can cause systemic hemodynamic instability, leading to decreased cardiac output and increased central venous pressure and hypoxemia. These factors can diminish and reduce glomerular filtration, potentially leading to kidney injury. Furthermore, several registries have highlighted a higher incidence of pulmonary embolism (PE) or venous thromboembolism (VTE) in patients with chronic kidney disease (CKD) (9, 10).

The present study aims to determine and evaluate the outcomes in patients with sPESI scores of 0, 1, and greater than 1, and to assess whether biomarkers such as BNP, TnI, and estimated glomerular filtration rate (GFR), along with right ventricular dysfunction on admission, enhance the predictive power of the sPESI score for risk stratification in acute pulmonary embolism (APE) patients.

METHODOLOGY

The data for this study were obtained from the Serbian multicentre PE registry, which consecutively included eight hospitals (seven university hospitals and one general hospital) from 2014 to 2020. Patients with pulmonary embolism (PE) were identified using and following the European Society of Cardiology (ESC) algorithm, with all diagnoses confirmed through positive findings on multidetector computed tomographic pulmonary (MDCT) angiography. Most patients were initially admitted to intensive care units for check-ups and evaluation. All participants provided oral informed consent for inclusion in the registry, and the study was carried out in accordance with the guidelines and principles of the Helsinki Declaration. The ethics committees of the participating university clinics approved the study.

Trained doctors responsible for managing the database recorded relevant data from the patients' medical history around the time of hospitalisation. Upon admission, they documented the patients' history of comorbidities, heart rate, oxygen saturation, as well as systolic arterial pressure.

Echocardiographic imaging was performed, and blood levels of cTnI, as well as BNP or NT-pro BNP (depending on the hospital), were measured on the first day of hospital admission. Before any treatment was initiated, peripheral venous blood samples were taken for creatinine testing upon hospitalisation. The samples were placed in standardized tubes with dipotassium ethylene dinitro tetra acetic acid (EDTA) and kept at room temperature. Measurements were carried out 30 minutes after the blood was collected. The measurement of creatinine levels was conducted using a method based on the rate-blanked Jaffe reaction technique (13). Renal function, or glomerular filtration rate (GFR), was calculated by using the Cockcroft-Gault formula:

(140–Age) x weight (kg) x F/serum creatinine (mmol/L), where F equals 1.23 for males and 1.04 for females. Based on the presence of severe hypotension and right ventricular dysfunction throughout their hospitalization, patients were categorized into three risk groups—high, intermediate, and low, following the 2019 ESC PE guidelines (4).

The sPESI score, used for evaluating risk in pulmonary embolism patients, accounts for various factors such as history of cancer, age over 80, chronic cardiopulmonary disease, heart rate of 110 beats per minute or more, systolic blood pressure less than 100 mmHg, and arterial oxygen saturation under 90% at diagnosis (14). Chronic cardiopulmonary disease encompasses conditions like chronic lung disease or heart failure. A diagnosis of heart failure is established based on the presence of the following factors: history of hospitalization for the condition, symptoms indicative of heart failure (New York Heart Association functional class above 2), or a left ventricular ejection fraction below 40%.

Chronic lung disease is characterized by persistent respiratory conditions, including restrictive lung diseases, asthma, and chronic obstructive pulmonary disease (COPD).

Patients are considered to have active cancer if they were treated for cancer within the previous six months (patients underwent curative or other types of surgery, received chemotherapy or immune therapy, had radiation procedures, or were treated with symptomatic therapy for cancer), are scheduled for cancer-related surgery, have metastases, or are diagnosed with terminal cancer with an estimated life expectancy of six months or less at the time of diagnosis.

Based on their sPESI scores, patients were categorized into three groups: group I consisted of patients with an sPESI score of 0; group II included those with an sPESI score of 1; group III comprised patients with an sPESI score greater of ≥ 2. All-cause mortality was tracked from the very first day of hospitalisation through the entire hospital stay.

Statistics

The patient data were expressed as frequencies for categorical variables and as the mean ± SD or the median with an interquartile range for numerical variables, depending on the data distribution. Differences between the two groups, based on in-hospital all-cause mortality, and among the three groups, categorized by sPESI score, were analysed using the Chi-square test or the Kruskal-Wallis test for independent samples. Unadjusted Cox regression models were employed to evaluate the predictive power of right ventricular dysfunction (RVD), BNP, TnI, and GFR, concerning the timing of all-cause mortality during hospitalization. Additionally, the predictive power of the sPESI score, categorized into three levels, was assessed.

RESULTS

The study examined 1,201 patients hospitalized for pulmonary embolism (PE), including 561 men and 640 women. The main patient characteristics are outlined in Table 1. Risk and prognostic factors were categorized into three main groups: medical history, clinical and laboratory findings upon admission, and PE severity according to the sPESI score, which also indicated mortality risk. During the hospital stay, 138 patients (11.5%) passed away, while 1,063 patients (88.5%) survived. The average duration of hospitalization was 11.5 ± 6.9 days, with a statistically significant difference between the lengths of stay for survivors and those who passed away (11.9 ± 6.5 days vs. 8.1 ± 8.7 days, $p < 0.0001$).

Higher mortality rates were significantly more associated with comorbidities like abnormal liver function and kidney injury ($p < 0.001$), as well as a history of cancer within the last six months ($p < 0.05$), coronary artery disease, diabetes, prior stroke, CHF, and COPD.

In the group of patients with the poorest outcomes, the clinical and laboratory findings indicated significantly higher BNP levels ($p < 0.05$), elevated heart rates on admission ($p < 0.01$), reduced oxygen saturation ($p = 0.001$), and lower systolic blood pressure on admission ($p < 0.001$). Additionally, a larger number of these patients had systolic blood pressure below 95 mmHg ($p < 0.001$), elevated TnI levels ($p < 0.001$), and increased right ventricular systolic pressure, indicative of right ventricular dysfunction.

Among the patients who passed away during their hospitalization, a higher sPESI score (> 1) was more commonly observed ($p < 0.001$), as anticipated. This group also had a greater proportion of patients classified as being at high risk of mortality ($p < 0.001$). There was also a statistically significant difference in all-cause mortality rates between the groups ($p < 0.0001$) (Table 1). In contrast, among the

Table 1. *Baseline patients' characteristics according to 30-day all-cause mortality*

	Hospital death		*p* value
	NO N = 1063 (88.5%)	**YES N = 138 (11.5%)**	
Female gender	559 (52.6)	81 (58.7)	0.2
Age in years	62.6±15.4	69.4 ± 14.5	0.07
MEDICAL HISTORY			
COPD	112 (10.5)	22 (15.9)	0.044
CHF	149 (14)	34 (24.6)	< 0.001
Coronary artery disease	118 (11.1)	116 (16.3)	0.006
Arterial hypertension	635 (59.7)	81 (58.7)	0.442
Prior stroke	64 (6.0)	20 (14.5)	0.001
Diabetes mellitus	187 (17.6)	37 (26.8)	0.008
Renal failure			
GFR < 60 ml/min	297 (27.9)	89 (64.5)	< 0.001
GFR < 30 ml/min	56 (5.3)	35 (25.4)	< 0.001
Abnormal liver function	38 (3.6)	19 (13.8)	< 0.001
Surgery within 6 months	159 (15)	20 (14.5)	**0.455**
History of cancer in last 6 months	133 (12.5)	27 (19.6)	0.017
Unprovoked PE	584 (54.9)	52 (37.7)	0.141
CLINICAL AND LABORATORY FINDINGS ON ADMISSION			
SaO_2 < 90%	938 (22.2)	122 (29.7)	0.001
SAP in mmHg	125±23	105 ± 30	< 0.001
SAP ≤ 95 mmHg	126 (11.9)	53 (38.4)	< 0.001
Heart rate in bpm	100±24	107 ± 27.8	0.01
RVSP[1] in mmHg	44.2±17.9	55.7 ± 16.0	< 0.001
BNP[2] in pg/ml	*127 (44-350)*	450 (44-350)	< 0.031
Troponin I[3] in μg/L	*0.05 (0.01-0.3)*	0.17 (0.05-0.9)	< 0.001
PE SEVERITY			
sPESI = 0	397 (37.3)	13 (9.4)	< 0.001
sPESI = 1	340 (32.0)	30 (21.7)	< 0.001
sPESI ≥ 2	326 (30.7)	95 (68.8)	< 0.001
PE mortality risk[4]			
Low risk	407 (38.3)	13 (9.4)	< 0.001
Intermediate risk	465 (43.7)	57 (41.3)	0.4
High risk	104 (9.8)	53 (38.4)	< 0.001
Hospital stay duration	11,9959 ± 6,56002	8,1207 ± 8,74758	< 0.0001

Abbreviations: IQR – interquartile range; COPD – chronic obstructive pulmonary disease; CHF – congestive heart failure; PE – pulmonary embolism; SaO_2 – oxygen saturation; SBP – systolic blood pressure; BPM– beats per minute; RVSP – right ventricular systolic pressure, GFR – glomerular filtration rate, 1RVSP was measured on admission in 1019 patients; BNP-brain natriuretic peptide, 2BNP was measured in 484; cardiac troponin I was measured in 690 patients; sPESI – simplified pulmonary embolism severity index. 4PE mortality risk was estimated for the time of entire hospitalization.

Table 2. *Biomarkers, estimated glomerular filtration rate, right ventricular dysfunction and 30-day all-cauze mortality according to three-level sPESI model*

	sPESI = 0 N 410	sPESI = 1 N 370	sPESI ≥ 2 N 421	p
GFR > 60, N = 792	322	258	212	< 0.001
GFR < 60, N = 403	85	111	207	< 0.001
TnI > 0.04, N = 467	135	138	194	< 0.001
TnI < 0.04, N = 467	150	91	83	< 0.001
BNP > 100, N = 501	114	161	226	< 0.001
BNP < 100, N = 264	153	72	39	< 0.001
RVDF, Y, N = 663	173	210	280	< 0.001
RVDF, Y, N = 413	212	116	85	< 0.001
L-Tx (%)	73 (17.8)	83 (22.4)	133 (31.6)	< 0.0001
Death PTE (%)	7 (1.7)	20 (5.4)	59 (14)	< 0.0001
Death N (%)	13 (9.4)	30 (21.7)	95 (68.8)	< 0.0001
Major bleeding (%)	32 (7.8)	45 (12.2)	34 (8.1)	ns

Abbreviations: BNP - brain natriuretic peptide; TnI - cardiac troponin I; GFR - glomerular filtration rate; RVD - right ventricular dysfunction; L-Tx – thrombolytic therapy; death PTE – pulmonary embolism as cause of death; death – all cause mortality

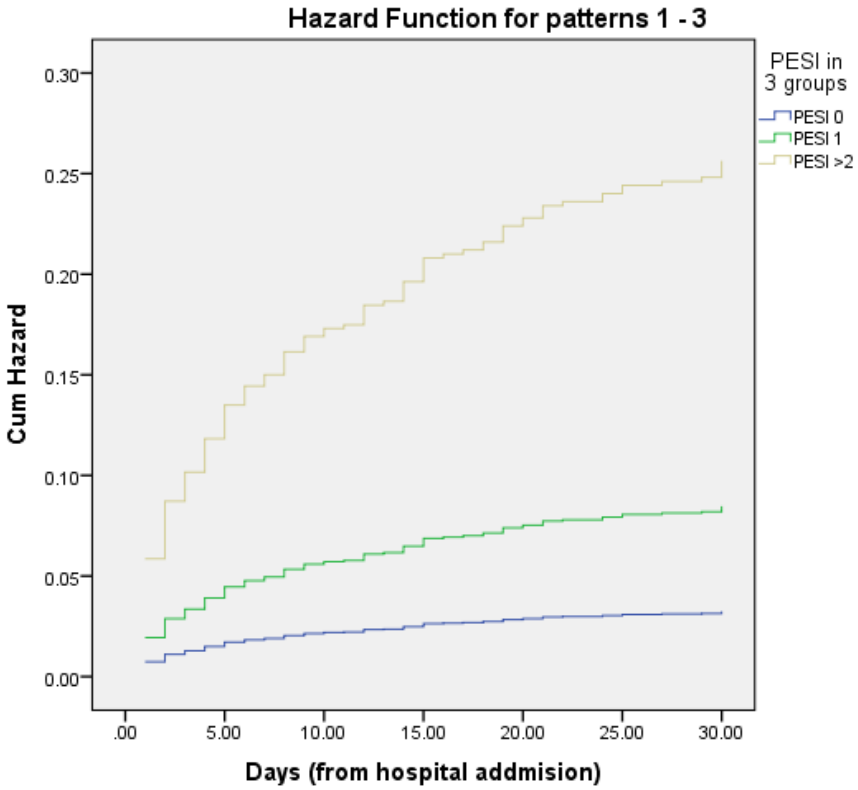

Figure 1. *Hazard for intrahospital all-cause death according to sPESI stratified into three subgroups sPESI 0, sPESI 1 and sPESI ≥ 2*

patients who survived, the distribution of sPESI scores (0, 1, and ≥ 2) was relatively even.

Patients were categorized into three subgroups based on their sPESI scores:

1. The sPESI 0 group included 410 patients.
2. The sPESI 1 group consisted of 370 patients.
3. The sPESI ≥ 2 group involved 421 patients.

The all-cause mortality rate and the mortality rate specifically due to pulmonary embolism differed significantly across the three groups categorized by sPESI scores ($p < 0.0001$) (Table 2). As anticipated, patients with sPESI scores greater than 1 were more often treated using systemic thrombolytics compared to those with sPESI scores of 1 or 0 ($p < 0.0001$).

An analysis of routine laboratory markers, including BNP, TnI, estimated GFR, and right ventricular dysfunction, showed significant variations across the groups with different sPESI scores (sPESI 0, sPESI 1, and sPESI ≥ 2) ($p < 0.001$). There were also notable differences in all-cause mortality rates among these groups, with hazard ratios indicating significant disparities [HR 0.127 (CI 0.071-0.226); $p < 0.0001$; HR 0.330 (CI 0.219-0.498); $p < 0.0001$]. Mortality rates specifically due to pulmonary embolism also varied significantly among all the groups [HR 0.113 (CI 0.052-0.247); $p < 0.0001$; HR 0.362 (CI 0.218-0.601); $p < 0.0001$] (Figure 1).

To identify additional parameters that could enhance the sPESI score, the Cox regression analysis was performed, including BNP, GFR, TnI, and right ventricular dysfunction across all three sPESI score groups (Table 3). The analysis revealed that GFR was the most effective predictor of all-cause in-hospital mortality [HR 2.24 (CI 1.264-3.969); $p = 0.006$] (Table 3) and mortality specifically due to pulmonary embolism (Figure 2). Right ventricular dysfunction [HR 1.608 (CI 0.977-4.203); $p = 0.262$], BNP levels [HR 0.733 (CI 0.288-1.866); $p = 0.514$], and TnI levels were not identified as significant predictors of in-hospital mortality independent of the sPESI score.

Table 3. *Hazard ratios for glomerular filtration rate, BNP, TnI, and RV dysfunction according to three-level sPESI score and 30-day all-cause mortality*

	HR	CI	p
GFR	2.24	1.264-3.969	0.006
BNP	0.733	0.288-1.866	ns
TnI	0.608	0.296-1.251	ns
RVD	1.687	0.677-4.203	ns

Abbreviations: BNP - brain natriuretic peptide; TnI - cardiac troponin I; GFR - glomerular filtration rate; RVD - right ventricular dysfunction

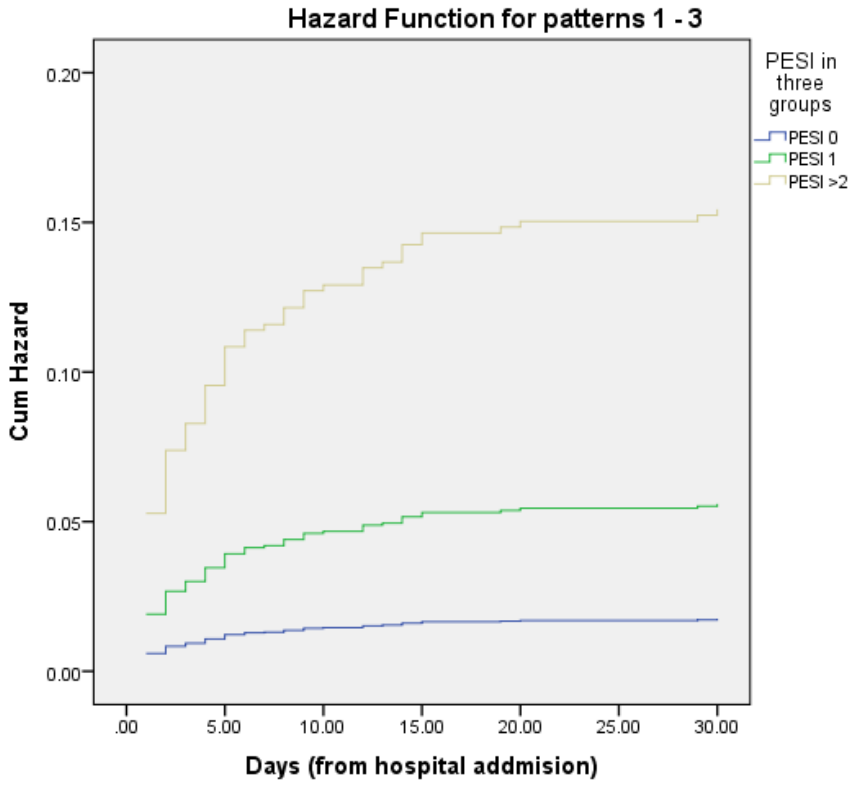

Figure 2. *Survival curve for pulmonary embolism as a cause of death according to sPESI stratified into three subgroups sPESI 0, sPESI 1, and sPESI ≥ 2*

DISCUSSION

The PESI score, whether in its original or simplified version, is frequently employed to assess the severity of acute pulmonary embolism (PE) because it integrates both comorbidities and clinical status parameters. PESI classes 0, I, and II are recognized as markers for low-risk PE. A meta-analysis encompassing 21 cohort studies with 3,295 patients diagnosed with "low-risk" PE based on PESI classes I-II or sPESI of 0 found that right ventricular (RV) dysfunction, detected via echocardiography or computed tomography pulmonary angiogram (CTPA), was present in 34% (95% CI 30-39%) of cases. An analysis of early mortality data from seven studies, which encompassed 1,597 patients, indicated an odds ratio (OR) of 4.19 (95% CI 1.39-12.58) for all-cause mortality when RV dysfunction and elevated cardiac troponin levels were present (15). Early all-cause mortality rates were relatively low compared to previous reports for intermediate-risk PE patients, with rates of 1.8% for RV dysfunction and 3.8% for elevated troponin levels (16). As a result, we incorporated RV dysfunction signs and elevated cardiac biomarkers to refine risk assessment within the intermediate-low-risk category, even among patients with a low PESI or sPESI of 0.

Our findings indicate that the three-tier sPESI model serves as an improved and more straightforward prognostic tool for risk assessment. Although sPESI 1 differs from sPESI 0, it may identify patients at higher risk for both in-hospital all-cause mortality and mortality specifically due to pulmonary embolism. Exclusively utilizing the sPESI score and GFR provides a rapid method for the prognostic stratification of acute PE patients, even at the time of hospital admission. Additionally, as previously noted, integrating clinical, biochemical, and imaging parameters with risk scores can enhance predictive accuracy. Troponin I, BNP, and right ventricular dysfunction are commonly employed for risk assessment and to guide treatment decisions. However, estimated GFR remains underutilized. While the glomerular filtration rate is often indicative of renal function, it can also reflect hemodynamic changes and alternations (17). Altinsoy B et al. published a study highlighting the significant role of GFR in pre-

dicting risk and its value for stratification in PE patients. They advocated for its inclusion in PE diagnostic and treatment guidelines (18).

Our study showed that estimated GFR was a strong prognostic marker for in-hospital all-cause mortality besides biomarkers such as TnI, BNP and RVD. Utilizing a multifaceted approach to risk estimation allows for the inclusion of eGFR in risk stratification, given its ease of use, availability, and reliability. Elevated serum creatinine levels may occasionally signal a decline in renal function. Despite this, eGFR remains a reliable marker for detecting deteriorating renal function in both acute and chronic kidney injuries, as well as in cardiovascular conditions. In cases of acute pulmonary embolism, hemodynamic disturbances may contribute to the onset of acute kidney injury (19).

Our findings suggest that incorporating GFR with the three-tier sPESI (0, 1, ≥ 2) enhances the prediction of in-hospital mortality. Previous studies have identified a GFR cutoff of 59 ml/min as optimal. Kostrubiec et al. also noted that a GFR below 60 ml/min on admission, with no subsequent improvement within three days, is indicative of a poor prognosis, with a 30-day mortality rate approximating 27% (20).

Limitations

While the three-tier sPESI model offers a straightforward method for risk stratification and prognosis, further internal and external validation is required. Additionally, a larger patient cohort that includes a diverse range of comorbidities and treatment strategies would be beneficial for increasing the accuracy of the model and supporting its broader acceptance.

CONCLUSION

The three-tier sPESI model (sPESI 0, sPESI I, and sPESI ≥ 2) represents a simple, efficient and straightforward prognostic tool for stratifying patients with acute pulmonary embolism. It effectively predicts both all-cause mortality and mortality specifically due to pulmonary embolism. The integration of GFR into the sPESI framework can further enhance its prognostic and therapeutic accuracy, potentially minimizing the need for additional biomarkers such as TnI, BNP, or indicators of right ventricular dysfunction in the risk assessment process and allowing for outpatient treatment.

Acknowledgements

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Conflict of interests

The authors declare that they have no conflict of interest.

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Poboljšanje stratifikacije rizika u plućnoj emboliji: integracija brzine glomerularne filtracije i pojednostavljenog indeksa težine plućne embolije kao snažnog prediktora preživljavanja bolesnika

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SAŽETAK

Uvod/Cilj. Kod bolesnika sa plućnom embolijom (PE) i pojednostavljenim indeksom ozbiljnosti plućne embolije (engl. *simplified pulmonary embolism severity index* – sPESI) 0 postoji nizak rizik od letalnog ishoda. Laboratorijski i ehokardiografski parametri, koji nisu uključeni u sPESI skor, mogu predstavljati prediktore nepovoljnog ishoda. Istraživanje je sprovedeno radi ispitivanja mogućeg poboljšanja prediktivne vrednosti sPESI skora uz pomoć vrednosti moždanog natriuretskog peptida (engl. *brain natriuretic peptide* – BNP), troponina (engl. *tropanin I* – TnI), ehokardiografskih parametara ili vrednosti glomeluralne filtracije (engl. *glomenular filtration rate* – GFR).

Metode. Ispitivanjem je obuhvaćen 1201 konsekutivni bolesnik sa potvrđenom plućnom embolijom. Na prijemu su svim bolesnicima urađeni ehokardiografski pregled, rutinske laboratorijske analize, TnI, BNP, kreatinin i GFR.

Rezultati. Intrahospitalni mortalitet bio je 11,5%. Bolesnici su podeljeni u tri grupe korišćenjem trostepenog sPESI modela: sPESI 0, sPESI 1 i sPESI ≥ 2. Postojale su statistički signifikantne razlike u mortatilitetu između triju grupa bolesnika, kao i vrednosti BNP-a, TnI-a, procenjene vrednosti glomenuralne filtracije (engl. *estimated glomenular filtration rate* – eGFR) i znakova disfunkcije desne komore (engl. *right venticular systolic dysfunction* – RVD). Prema Coxovoj regresionoj analizi, najbolji prediktor tridesetodnevnog ukupnog mortliteta bio je eGFR [HR 2,24 (CI 1,264–3,969); p = 0,006] u svim trima grupama. Korišćenjem

trostepenog sPESI modela zaključili smo da TnI, BNP ili RVD nisu doprineli poboljšanju stratifikacije rizika.
Zaključak. Renalna disfunkcija na prijemu bolesnika sa plućnom embolijom udružena je sa visokim rizikom od intrahospitalnog mortaliteta. Trostepeni sPESI model može se koristiti sa ciljem prognostičke stratifikacije bolesnika sa akutnom plućnom embolijom.

Ključne reči: **pulmonalna, embolija, skor pojednostavljenog indeksa ozbiljnosti plućne embolije, prognoza**

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Original article

Association of Serum Zinc and Selenium Concentration with Insulin Resistance in Apparently Healthy Adults

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SUMMARY

Introduction/Aim. Zinc is a trace element involved in insulin metabolism, including its production, storage, and release. Selenium is regarded as a vital micronutrient for humans. It participates in insulin signaling and control. Zinc and selenium may be possibly linked to insulin resistance; however, these relationships have not been well investigated. Therefore, we sought to examine the relationship between blood zinc and selenium levels and insulin resistance in apparently healthy individuals.
Methods. This study used a cross-sectional design including 203 apparently healthy people. Measurements were taken to determine zinc and selenium serum levels, fasting insulin, fasting blood glucose, and glycosylated hemoglobin. Insulin resistance was measured by utilizing the Homeostatic Model Assessment (HOMA–IR).
Results. The prevalence of insulin resistance, as determined by HOMA-IR, was 26.11%. Patients with insulin resistance had higher age (59.96 ± 12.28 years), body mass index (26.66 ± 3.16 kg/m^2), and waist-to-hip ratio (0.93 ± 0.05) compared to those with insulin sensitivity (54.19 ± 9.88 years, 25.92 ± 2.4 kg/m^2, 0.91 ± 0.05), with statistically significant differences (p-values—0.013, 0.013, 0.029, respectively). Serum zinc levels were elevated in insulin-sensitive individuals (87.12 ± 6.87 mcg/mL) compared to those who were insulin-resistant (84.05 ± 8.29 mcg/mL), with a p-value of 0.036. HbA1c concentration and fasting insulin levels were elevated in the insulin-resistant group (4.95 ± 0.49, 15.78 ± 1.59) compared to the insulin-sensitive group (4.79 ± 0.38, 10.1 ± 2.34), with p-values of 0.033 and 0.003, respectively.
Conclusion. In apparently healthy adults, there is an association between low serum zinc levels and insulin resistance. There is no association between selenium serum levels and insulin resistance.

Keywords: **insulin resistance, zinc, selenium**

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INTRODUCTION

The association between zinc content and insulin secretion and function was identified in 1930 as the zinc ion was found to stimulate insulin crystallization (1). Further research demonstrated that zinc is necessary for the processing and storing insulin in the pancreatic β-cell. Zinc (Zn) helps the pancreas produce and store insulin, which augments the body's absorption of glucose. Low plasma levels of zinc impair islet cell production and secretion of insulin (2). Pancreatic β-cells store and crystallize insulin in granules along with free cytosolic zinc. Under normal conditions, β-cells form a hexamer by combining six insulin monomers with two zinc ions in the core. The insulin-Zn crystal is stored and transferred across the cell membrane. According to a study in 2015 by Slepchenko et al., only the insulin monomer remains an active form of the hormone after the Zn-insulin complexes dissociate after being secreted (3). The increase in insulin secretion is correlated with the elevated extracellular concentration of free zinc (3).

Selenium (Se) is a vital mineral for the proper function of human organs. Selenium can be found in both organic and inorganic forms in nature. Selenate, selenite, selenide, and elemental selenium are examples of the inorganic forms of selenium. In contrast, the organic form is found in combination with amino acids and is referred to as selenomethionine (SeMet) and (selenocysteine) SeCys (4). Since selenium counteracts the effects of insulin through glutathione peroxidase (GPx-1) and selenoprotein p (SelP), elevated plasma selenium concentrations are linked to diabetes biomarkers (5). Pan-creatic β-cells are protected from oxidative stress by the action of enzymes like GPx, catalase, and superoxide dismutase, peroxiredoxins, thioredoxins, and thioredoxin reductases. Since the bioavailability of selenium is essential for the activity of thioredoxin reductases and GPx, selenium deficiency causes β-cell oxidative damage and decreased insulin secretion. However, too much selenium also causes dysregulation of insulin secretion, which raises insulin levels and results in the T2DM phenotype. These antioxidant enzymes, including selenoenzymes, generally interfere with vital redox signaling for cell differentiation and insulin secretion to influence these processes (6).

HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) is a practical quantification method for assessing insulin resistance from an epidemiological or clinical perspective. Nevertheless, significant diversity exists in its threshold values. Furthermore, these threshold values vary based on ethnicity, clinical evaluation methodologies, and the metabolic state of the tested populations (7). The HOMA-IR thresholds for diagnosing insulin resistance may differ among racial groups (8). A recent study involving 1,327 non-diabetic, normotensive people in Tehran established this threshold at 1.8. Certain investigators have attempted to identify HOMA-IR cut-offs in individuals predisposed to insulin resistance or metabolic syndrome, although their results were inconsistent (9).

In our investigation, the HOMA-IR threshold was determined to be 3.1, aligning with prior studies on healthy Iraqi adults. Higher than this, individuals were classified as having insulin resistance (10, 11). The relationship between zinc, selenium, and insulin resistance in healthy individuals is intricate. Although research links zinc insufficiency to insulin resistance, selenium's effects are multifaceted, revealing positive and negative associations. The role of zinc and selenium in insulin resistance is still a matter of discussion. In this study, we try to explain the association of serum zinc and selenium with insulin resistance in apparently healthy adults.

SUBJECTS AND METHODS

The research included 203 apparently healthy Iraqi individuals of both genders who were identified as Arabs and were ≥ 40 years old. This cross-sectional study was conducted from May 2023 to February 2024. The study was conducted at Al Nahrain University/College of Medicine, Al-Farahidi University, and Mustansiriyah University, Baghdad, Iraq. It was approved by the Institutional Review Board (IRB) of the College of Medicine, Al-Nahrain University, under the reference number 20221027 on 01/02/2023. Prior to participating in the study, all participants provided their written informed consent.

Inclusion criteria

Subjects enrolled in the study were apparently healthy adults ≥ 40 years old, euglycemic, non-diabetic with HbA1C ≤ 6.5%.

Exclusion criteria

The presence of diabetes or other severe chronic disease such as heart and renal diseases, as well as the use of medications may influence IR or lipid metabolism (such as corticosteroids and lipid-lowering drugs), pregnancy, breastfeeding and women receiving oral contraceptive or hormone replacement therapy.

Sample collection: Participants were asked to fast for eight hours prior to samples' collection. The next morning, 5 ml of blood was taken from each participant. Blood samples were placed in a gel tube to collect serum. After that, the serum was recovered by centrifuging the sample for 15 minutes at 4000 rpm×g. The serum was collected and poured into many Eppendorf tubes for biochemical testing. Two milliliters of blood were obtained in two ethylene diamine tetra-acetic acid (EDTA) tubes for hematological tests.

Demographic factors: Demographic variables such as age, gender, smoking habits, place of residence, and family history of diabetes were obtained by direct interview. The participant's body weight (in kilograms) and height (in centimeters) were measured under the condition that they were wearing light clothes and without wearing shoes. The body mass index was determined using the following equation: weight (kg)/square height (m^2). Measurements were taken for waist circumference, hip circumference, and waist-to-hip ratio.

Biochemical tests: Using a commercially available kit, the hexokinase technique was used to assess glycosylated hemoglobin and fasting plasma glucose. Fasting insulin was determined by immunoradiometric assay. The calculation of insulin resistance was performed using the HOMA-IR method, as follows: HOMA-IR = (FBS (mg/dl) x fasting insulin (mU/L) / 405. In our investigation, the HOMA-IR cut-off value was established at 3.1, consistent with other research involving apparently healthy Iraqi adults. Higher than this, individuals were classified as having insulin resistance (10, 11).

Serum level of zinc: A colorimetric method with 5-bromo-PAPS was used to measure the serum zinc concentration. The assay principle involves the formation of a chelate complex between zinc and 2-(5-bromo 2-pyridylazo)-5(N-propyl-N- sulfopropyl-amino)-phenol. The rise in absorbance may be quantified and is directly proportional to the quantity of total zinc in the sample.

The normal range for males is 72.6-127 mg/dL, while in females it is 70.6-114 mg/dL.

Serum level of selenium: The serum level of selenium was measured by an atomic absorption spectrophotometer, which examined the wavelength of photons absorbed during the excitation of element atoms. The technique used to estimate the atomic absorption of elements is the graphite furnace method, in which the approximation reaches the limit of concentrations in parts per billion. The sample was placed in a graphite tube inside the electric furnace, evaporating until dry, burned, and converted to the atomic state. Here, the percentage of atoms that evaporated, decomposed, and became ready to absorb energy is more significant than in the case of direct flame. The results appeared directly on a screen linked to an atomic absorption spectrophotometer.

Statistical analyses: Statistical analyses were conducted using the SPSS software version 25.0 (SPSS, Chicago). The continuous data underwent a normality test using the Shapiro-Wilk test. Data that exhibited a normal distribution were reported as the mean and standard deviation and were analyzed using a Student's t-test. Data that did not follow a normal distribution were reported using the median and range, and were evaluated using the Mann-Whitney U test (for comparing two groups). Categorical variables were quantified using numerical values and percentages and then assessed using the Chi-square test. Participants were categorized into two groups based on whether they had insulin resistance or not according to HOMA-IR. In line with the previous population-based studies, the cutoff value of HOMA-IR was determined to be 3.1, beyond which subjects were considered to have IR (10, 11).

RESULTS

Demographic features of the study population

Table 1 displays the age, sex, weight, height, BMI, waist and hip circumferences, and the waist/hip ratio of the population under investigation.

Table 1. *Demographic data of the study population*

Parameter	Value
Age, years	
Mean ± SD	55.55 ± 10.75
Range	40-82
Sex	
Males	108 (53.2%)
Females	95 (46.8%)
Weight (Kg)	
Mean ± SD	72.01 ± 8.15
Range	47-89
Height, cm	
Mean ± SD	168.63 ± 9.71
Range	151-182
Waist circumference, cm	
Mean ± SD	89.42 ± 7.13
Range	70-103
Hip circumference, cm	
Mean ± SD	96.34 ± 7.91
Range	80-106
Body Mass Index, kg/cm^2	
Mean ± SD	26.09 ± 2.61
Range	20.34-34.6
Waist/Hip ratio	
Mean ± SD	0.92 ± 0.05
Range	0.77-1.02

Table 2. *Glycemic profile, insulin level, and HOMA-IR of the study population*

Parameter	Value
Fasting blood glucose, mg/dL	
Mean ± SD	88.49 ± 6.80
Range	71.4-109
Glycosylated hemoglobin, %	
Mean ± SD	4.83 ± 0.42
Range	4-5.9
Fasting insulin, μU/mL	
Mean ± SD	11.44 ± 3.26
Range	5.4-19.5
HOMA-IR	
Mean ± SD	2.5 ± 0.71
Range	1.17-4.02

The fasting blood glucose, HbA1c, and insulin levels and the HOMA-R of the study population are indicated in Table 2.

Table 3 shows serum zinc and selenium plasma levels for the studied population.

Table 3. *Trace elements of the study populations*

Parameter	Value
Zinc, mcg/mL	
Mean ± SD	86.11 ± 6.88
Range	71.5-101.5
Selenium, μg/L	
Mean ± SD	0.29 ± 0.15
Median	0.26
Range	0.06-0.74

Insulin status

The study population was divided into insulin-resistant or not based on HOMA IR. One hundred and fifty (73.89%) subjects were insulin sensitive, and the rest, 53 (26.11%) subjects, were insulin resistant (Figure 1).

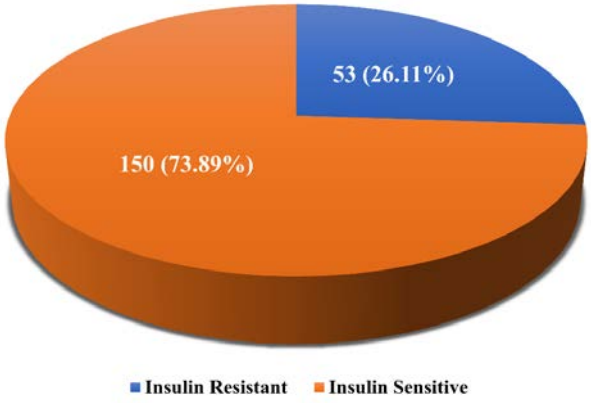

Figure 1. *Distribution of the study population into insulin-sensitive and insulin-resistant subjects*

Demographic data according to insulin status

Subjects of the insulin-resistant group were older and had higher BMIs than those of the insulin-sensitive group (p = 0.013, p = 0.013, respectively). Similarly, the WHR was higher in the insulin resistant group than the insulin-sensitive group (p = 0.029). Conversely, no significant difference was demonstrated in weight, height, waist, and hip circumferences. Likewise, no sex difference was noticed between the two subgroups, as shown in Table 4.

Insulin levels according to insulin resistance status

When subjects were divided into two groups, the HbA1c levels were 4.95 ± 0.49% versus 4.79 ± 0.38%, P = 0.033. The fasting insulin levels were 15.78 ± 1.59 μU/mL versus 10.1 ± 2.34 μU/mL (P = 0.003). FBG levels were 89.04±6.88 mg/dL versus 88.32 ± 6.78 mg/dL (P = NS). Thus, subjects with IR appeared to have higher levels of HbA1c insulin levels compared with those with insulin sensitivity but retained FBG levels, as shown in Table 5. For the p-value, the t-test was used.

Serum trace elements according to insulin status

Table 6 presents the level of trace elements in IR compared to insulin-sensitive subjects. Zinc serum level was 84.05 ± 8.29 mcg/mL versus 87.12 ± 6.87 mcg/mL (P = 0.036) respectively, whereas selenium level was 0.26 ± 0.11 ppm versus 0.30 ± 0.16 ppm (P = NS).

Table 4. *Demographic data of the study population according to insulin status*

Parameter	Insulin status		p-value
	Resistant (n = 53)	Sensitive (n = 150)	
Age, years			
Mean ± SD	59.96 ± 12.28	54.19 ± 9.88	0.013
Range	70-82	40-82	
Sex			
Males	31(58.5%)	77 (51.33%)	0.506^{NS}
Females	22 (41.5%)	73 (48.67%)	
Weight (kg)			
Mean ± SD	72.88 ± 7.56	71.75 ± 8.33	0.775 ^{NS}
Range	55-85	47-89	
Height, cm			
Mean ± SD	170.1 ± 5.82	168.17 ± 6.92	0.250 ^{NS}
Range	159-182	151-182	
Waist circumference, cm			
Mean ± SD	92.1 ± 6.28	88.59 ± 7.18	0.244 ^{NS}
Range	77-103	70-102	
Hip circumference, cm			
Mean ± SD	97.81 ± 4.17	95.88 ± 5.04	0.133 ^{NS}
Range	84-106	80-105	
Body mass index, kg/m^2			
Mean ± SD	26.66 ± 3.16	25.92 ± 2.4	0.013
Range	20.4-34.2	20.34-34.6	
Waist/Hip ratio			
Mean ± SD	0.93 ± 0.05	0.91 ± 0.05	0.029
Range	0.79-1.02	0.77-0.98	

NS = not significant. P-value for all parameters, we used t-test except for the p-value for sexes; we used the Chi-square test

Table 5. *Insulin resistance-related parameters of those with and without insulin resistance*

Parameter	Insulin status		p-value
	Resistant (n = 53)	Sensitive (n = 150)	
FBG, mg/dL			
Mean ± SD	89.04 ± 6.88	88.32 ± 6.78	0.305^{NS}
Range	77-109	71.4-106	
HbA1c level, %			
Mean ± SD	4.95 ± 0.49	4.79 ± 0.38	0.033
Range	4-5.9	4-5.7	
Fasting insulin, μU/mL			
Mean ± SD	15.78 ± 1.59	10.1 ± 2.34	0.003
Range	12.5-19.5	5.4-16.5	

FBG = fasting blood glucose; HbA1C = glycosylated hemoglobin; NS = not significant
For the p-value, the t-test was used

Table 6. *Serum trace elements according to insulin status*

Parameter	Insulin status		p-value
	Resistant (n=53)	Sensitive (n=150)	
Zinc, mcg/mL			
Mean ± SD	84.05±8.29	87.12±6.87	0.036
Range	71.5-101.5	74.1-101.4	
Selenium, ppm			
Mean ± SD	0.26±0.11	0.30±0.16	0.222[NS]
Range	0.06-0.74	0.06-0.74	

NS = not significant
For the p-value, the t-test was used

DISCUSSION

The present study showed that the serum zinc was lower in insulin-resistant subjects than in insulin-sensitive subjects. Many studies worldwide have confirmed this finding. Research conducted in Bangladesh found a correlation between the zinc content in the blood and insulin resistance in 142 individuals with normal blood sugar levels (12). Research conducted by Ahn et al. in Korea showed that there is a negative correlation between the concentration of zinc in the blood and insulin resistance in a large group of adults without diabetes (13).

A review study involving 56 articles found that zinc deficiency is associated with glucose intolerance and IR; however, the effectiveness of the intervention with the zinc supplementation is still inconclusive (14). In a separate study undertaken by Bjørklund et al., it was shown that zinc, selenium, and copper have a role in the development of diabetes (15). However, these trace elements are in excessive quantities and have harmful effects. Zinc seems to stimulate essential molecules involved in cellular signaling, which regulate glucose homeostasis. Zinc also modulates insulin receptors, extends the duration of insulin activity, and enhances favorable lipid profiles (15).

A recent experimental investigation explored the impact of zinc on insulin levels in male rats. The study shows that levels of zinc in the food influence the levels of insulin in the bloodstream and change the distribution of pancreatic β-cells responsible for producing insulin. Their findings indicate that variations in blood insulin levels, triggered by varying plasma concentrations of zinc, may lead to metabolic changes in insulin target organs, such as the liver and adipose tissue (16).

Multiple further investigations have shown that zinc plays a crucial role in the pathophysiology of glucose metabolism and affects insulin homeostasis (17). Previous research showed that persons with impaired glycemic control had considerably lower serum levels of zinc than healthy individuals (18). Furthermore, there is an evidence indicating that decreased levels of zinc are linked to a higher likelihood of acquiring type 2 diabetes (T2D) in the future (19).

Multiple publications indicate the processes by which zinc is implicated in insulin action. Zinc acts as a catalyst in promoting the process of phosphorylation of the beta component of the insulin receptor. The activity of phosphoinositide 3-kinases (PI3K) protein kinase B (PKP) mediates the insulin-like action on glucose transport (20). Zinc exerts its insulin-like effects by directly inhibiting endogenous glycogen synthase kinase-3beta (GSK-3β), a protein linked to insulin resistance (IR) and type 2 diabetes (21).

Another mechanism involves the interaction with oxidative stress, which has a role in developing and advancing insulin resistance (IR) and diabetes (22, 23). It is well established that pancreatic β cells are susceptible to damage from free radicals. Zinc, a cofactor of superoxide dismutase, reduces oxidative stress. Therefore, a zinc deficiency might worsen oxidative stress and contribute to the development of insulin resistance (24). Furthermore, zinc transporter 8 (ZnT8), situated on the surface of pancreatic β-cells, plays a crucial role in zinc's appropriate storage and functioning in insulin-secretory granules (25). The impairment of ZnT8 function or genetic mutations in the ZnT8 gene hinders the normal process of insulin crystallization. This results in increased degradation of insulin in the liver, decreasing the quan-

tity of insulin that reaches the target tissues. Consequently, this leads to the development of impaired glucose tolerance (26). The correlation between selenium and insulin resistance (IR) in animals and people is still unclear due to conflicting findings from prior research. Some studies indicate that an excessive buildup of selenium in the body is linked to type 2 diabetes (27), whereas other investigations do not uncover any significant connections (28). Research indicates that optimal levels of selenium are crucial for the production and effectiveness of insulin. However, an excessive amount of selenium in the body is linked to the development of insulin resistance (IR) and diabetes mellitus (DM) (29).

An extensive cross-sectional investigation on a representative sample of the U.S. population found a direct correlation between the levels of selenium in the blood and diabetes (30). The distinction in this research is that selenium was evaluated in plasma, which serves as a short-term indicator and is more responsive to immediate dietary fluctuations. Moreover, the study relied on a physician's self-reported diagnosis of diabetes, which might introduce bias and lead to an overestimation of the prevalence of diabetes. In contrast, a cross-sectional analysis of the health professionals' research revealed a negative correlation between toenail selenium levels and the incidence of diabetes among male health professionals (31). Zinc and selenium are both crucial for the metabolism of glucose and insulin. However, further studies are required to corroborate these results and clarify the underlying mechanisms.

CONCLUSION

The study indicates an association between apparently healthy adults' serum zinc levels and insulin resistance. There is no association between selenium concentration and insulin resistance. Additional longitudinal or experimental studies may be required to ascertain a causal relationship.

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Povezanost koncentracija cinka i selena u serumu sa insulinskom rezistencijom kod naizgled zdravih odraslih osoba

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SAŽETAK

Uvod/Cilj. Cink je element koji je u tragovima uključen u metabolizam insulina, uključujući proizvodnju, skladištenje i oslobađanje insulina. Selen se smatra vitalnim mikronutrijentom kod ljudi i učestvuje u signalizaciji i kontroli insulina. Cink i selen mogu biti povezani sa insulinskom rezistencijom. Međutim, ovi odnosi još nisu dobro istraženi. Iz tog razloga, nastojali smo da ispitamo odnos između nivoa cinka i selena u krvi i insulinske rezistencije kod naizgled zdravih osoba.
Metode. U ovoj studiji primenjen je poprečni presek, a u istraživanje su uključene 203 naizgled zdrave osobe. Merenja su izvršena da bi se odredili nivoi cinka i selena u serumu, insulin natašte, glukoza u krvi natašte i glikolizirani hemoglobin. Insulinska rezistencija merena je korišćenjem procene homeostatskog modela (engl. *homeostasis model assessment for insulin resistance* – HOMA-IR).
Rezultati. Prevalencija insulinske rezistencije, kako je utvrđeno modelom HOMA-IR, iznosila je 26,11%. Bolesnici sa insulinskom rezistencijom bili su stariji (59,96 godina ± 12,28 godina), imali su viši indeks telesne mase (26,66 kg/m² ± 3,16 kg/m²), kao i povećanu vrednost odnosa struka i kuka (0,93 ± 0,05), nego ispitanici osetljivi na insulin (54,19 godina ± 9,88 godina, 25,92 kg/m² ± 2,4 kg/m², 0,91 ± 0,05), sa statistički značajnim razlikama (p vrednosti: 0,013, 0,013, 0,029, redom). Nivoi cinka u serumu bili su povišeni kod osoba osetljivih na insulin (87,12 mcg/mL ± 6,87 mcg/mL) u poređenju sa osobama koje su bile otporne na insulin (84,05 mcg/mL ± 8,29 mcg/mL), sa p vrednošću od 0,036. Koncentracija HbA1c, kao i nivoi insulina natašte, bili su povišeni u grupi ispitanika rezistentnih na insulin (4,95 ± 0,49, 15,78 ± 1,59) u poređenju sa grupom ispitanika osetljivih na insulin (4,79 ± 0,38, 10,1 ± 2,34), sa p vrednostima od 0,033 i 0,003, redom.
Zaključak. Kod naizgled zdravih odraslih osoba utvrđena je povezanost između niskog nivoa cinka u serumu i insulinske rezistencije. Ne postoji povezanost između nivoa seruma selena i insulinske rezistencije.

Ključne reči: **insulinska rezistencija, cink, selen**

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Original article

Predictive Parameters of Arteriovenous Fistula Maturation for Hemodialysis in Patients with Diabetes Mellitus

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SUMMARY

Introduction/Aim. Arteriovenous fistula (AVF) is recommended as the ideal vascular access for hemodialysis (HD), however, there are conflicting opinions when it comes to patients with diabetes mellitus (DM). The aim of the paper was to determine the predictive parameters of AVF maturation for HD in patients with DM.

Methods. The investigation was organized as a retrospective, descriptive-analytical study. The target group of our research involved 209 patients with DM, in whom AVF was created for HD. We recorded demographic and gender characteristics, location and type of AVF at the time of creation, type of anastomosis, data on the initial (a)function of the fistula, HD catheter placement, and blood pressure. Before the operation, Doppler ultrasound of the blood vessels was performed, and intraoperatively, the lumen of the artery and vein used to form the AVF was measured. We analyzed laboratory variables that were routinely controlled in our institution.

Results. Diabetics with successful maturing fistula significantly more often had proximally located AVF (p = 0.004), end-to-side anastomosis type (p = 0.036), and initial function (p = 0.001). In a univariate analysis, the brachiocephalic location of AVF (p = 0.004), end-to-side type of anastomosis (p = 0.039), and initial function of AVF (p = 0.001) were the predictive parameters of AVF maturation. Multivariable statistical analysis showed that brachiocephalic localization of AVF (p=0.021), end-to-side anastomosis type (p = 0.004), and initial function of AVF (p = 0.001) are the predictive parameters of AVF maturation in diabetics.

Conclusion. Predictive parameters of fistula maturation, in patients with DM in our study, are the initial function of AVF, brachiocephalic location, and end-to-side anastomosis.

Keywords: **arteriovenous fistula, hemodialysis, diabetes mellitus, maturation, predictive parameters**

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INTRODUCTION

In 2021, 529 million people were living with diabetes worldwide. By 2050, more than 1.31 billion people are projected to have diabetes (1).

Among the numerous health consequences of diabetes mellitus, one of the microvascular complications is diabetic nephropathy, which is chronic, progressive, and irreversible and leads to end-stage renal disease. However, it should be emphasized that in the early stages of diabetic nephropathy (stage I, stage II, and partially stage III), microvascular changes are less pronounced (2).

From the options offered, arteriovenous fistula is the most preferred option of vascular access for chronic hemodialysis and it is designated by guidelines and initiatives as the "best" available vascular access for hemodialysis. However, even though many authors have such a view, there are opposing opinions, because diabetes mellitus may be associated with arteriovenous fistula failure, most likely due to atherosclerosis, which is more pronounced in patients with diabetes, with a wide range of vascular lesions, making it difficult to establish vascular access. Diabetes is often accompanied by hyperlipemia, hypoalbuminemia, and high blood coagulation, which also leads to arteriovenous obstruction. Also, due to hemodynamic effects, the vein near the anastomotic stoma is struck by blood flow, leading to inner membrane injury, platelets, and fiber deposition, causing vascular intimal hyperplasia and stenosis (3, 4).

Yet, Gordon et al. (5) advocate the use of arteriovenous fistula in patients with diabetes, emphasizing its benefits despite various complications that may arise.

Anyway, the ultimate goal of angioaccess surgery is functional, mature, and durable access with a low complication rate. The downside is that there are many complications, mainly related to thrombosis when the arteriovenous fistula is applied, so complications such as thrombosis of vascular access remain the main problem for many patients with kidney failure, especially as a consequence of diabetes mellitus (6).

The aim of the study was to determine the predictive parameters of maturation of an arteriovenous fistula for hemodialysis, in patients with diabetes mellitus.

METHODOLOGY

The study was organized as a retrospective, descriptive-analytical study at the Clinic for Nephrology and Dialysis of the University Clinical Center in Kragujevac, Serbia.

Over a fifteen-year period, we created arteriovenous fistulas in 1,202 patients for the treatment of end-stage renal failure. The target group of our interest consisted of 209 subjects who, as the etiology of renal failure, had diabetes mellitus.

Among them, there were 148 (70.8%) subjects with a mature arteriovenous fistula, and 61 (29.2%) subjects had an immature fistula.

Maturation of arteriovenous fistulas was defined as access use for effective dialysis using two needles for 75% or more dialysis sessions over four weeks (7). A fistula is mature when it can be, nine months after arteriovenous fistula creation, routinely cannulated with two needles and delivered a minimum blood flow (typically 350–450 ml/min) for the total duration of dialysis, usually 3–5 h for high efficiency hemodialysis (8).

From our database, we recorded the demographic and gender characteristics of the patients, the location of the arteriovenous fistula (distally or proximally located), information on whether the arteriovenous fistula was created before starting hemodialysis, the type of anastomosis (end-to-side or end-to-end), information on hemodialysis catheter placement, systolic and diastolic blood pressure components, mean arterial blood pressure, and data on the initial (a)function of the fistula. In all subjects, the lumen of the artery and vein was measured intraoperatively for arteriovenous fistula formation. Likewise, we recorded the preoperative use of Doppler ultrasound. An ultrasound examination was performed on a Shimadzu SDU-2200, Tokyo, Japan, using Doppler B mode ultrasonography and a 7.5 MHz resolution probe.

We analyzed laboratory variables (erythrocytes, leukocytes, hemoglobin, platelets, albumins, glucose, uric acid, calcium, phosphorus, cholesterol, triglycerides, high-density lipoprotein, low-density lipoprotein, fibrinogen), which were performed before surgery, which are routinely controlled in our institution for all hospitalized patients and which, according to our experience, would affect the maturation of the arteriovenous fistula for hemodialysis.

The study was approved by the Ethics Committee of the Clinical Center Kragujevac, by the Helsinki Declaration for Medical Research.

Statistical methods

The data were analyzed using SPSS for Windows, version 19. The level of statistical significance was set at $p \leq 0.05$. The variables were described using relative numbers, central tendency, and variability measures. The Mann-Whitney test and the Chi-square test were used to evaluate differences in the parameters under investigation. To examine associations of independent variables of interest with arteriovenous fistula maturation among diabetic patients, we performed a multivariable logistic regression analysis of factors associated with arteriovenous fistula maturation in the univariate regression analysis.

RESULTS

The study comprised 209 hemodialysis patients diagnosed with diabetes mellitus. The majority of patients–142 (67.9%) were male, and the average age of the patients was 60.77 ± 10.77 years (mean standard deviation-SD). Females were on average 63 ± 61.08 years old, while males were on average 62 ± 60.8 years old. Mature fistulas were recorded in 148 (70.8%) subjects, proximally located (brachiocephalic) fistulas were observed in 134 (64.1%) subjects, 175 (83.7%) patients had end-to-side anastomosis, and 108 (51.7%) subjects had arteriovenous fistulas created after the start of hemodialysis. Doppler was performed in 105 (50.2%) patients, and a central venous catheter was placed in 89 (42.6%) patients. The initial function of the arteriovenous fistula was recorded in 142 (67.9%) patients, while in 67 (32.1%) subjects the arteriovenous fistula was a primary failure.

Diabetics with a functional arteriovenous fistula significantly more often had a proximally located arteriovenous fistula (brachiocephalic), end-to-side type of anastomosis, and initial function of the arteriovenous fistula (Table 1).

In a univariate analysis, the brachiocephalic location of arteriovenous fistula ($p = 0.004$), end-to-side type of anastomosis ($p = 0.039$), and initial function of arteriovenous fistula ($p = 0.001$) were predictive parameters of arteriovenous fistula maturation (Table 2).

Multivariable statistical analysis showed that brachiocephalic localization of arteriovenous fistula ($p = 0.021$), end-to-side type of anastomosis ($p = 0.004$) and initial function of arteriovenous fistula ($p = 0.001$) are the predictive parameters of arteriovenous fistula maturation in diabetics (Table 3).

Table 1. *Sociodemographic and clinical characteristics of the subjects*

Variables		Matured AVF n (%) = 148 (70.8)	Immature AVF n (%) = 61 (29.2)	p-value
		Mean ± SD	Mean ± SD	
Age		60.94 ± 11.13	60.34 ± 9.91	0.468
Gender n (%)	Male	105 (73.9)	37 (26.1)	0.147
	Female	43 (64.2)	24 (35.8)	
ESR		65.76 ± 35.23	63.87 ± 37.60	0.754
RBC		3.15 ± 0.44	3.24 ± 0.49	0.364
WBC		8.72 ± 3.19	8.29 ± 2.50	0.456
HGB		92.51 ± 12.29	92.75 ± 17.42	0.609
PLT		235.10 ± 80.54	250.77 ± 88.1	0.239
Alb		33.54 ± 6.89	31.94 ± 7.22	0.113
Glu		7.50 ± 3.46	7.24 ± 3.48	0.565
Uric acid		424.99 ± 112.86	433.46 ± 141.93	0.935
Ca		2.10 ± 0.26	2.10 ± 0.21	0.891
PO_4		1.71 ± 0.54	1.61 ± 0.56	0.100
Systolic blood pressure (mmHg)		158.35 ± 30.60	154.17 ± 28.35	0.193
Diastolic blood pressure (mmHg)		86.80 ± 25.55	80.34 ± 12.24	0.239
MEAN blood pressure (mmHg)		106.12 ± 21.84	107.88 ± 23.90	0.978
Cholesterol		5.01 ± 2.25	4.85 ± 1.91	0.909
Trig		2.18 ± 1.23	2.21 ± 1.34	0.987
HDL		0.98 ± 0.36	1.10 ± 0.43	0.232
LDL		3.07 ± 1.99	3.08 ± 2.18	0.855
Fibrinogen		5.29 ± 2.47	5.03 ± 1.49	0.880
Intraoperative vein diameter (mm)		2.28 ± 0.30	2.28 ± 0.45	0.550
Intraoperative artery diameter (mm)		2.40 ± 0.41	2.31 ± 0.53	0.649
Location of AVF n (%)	Radiocephalic	44 (58.7)	31 (41.3)	*0.004
	Brachiocephalic	104 (77.6)	30 (22.4)	
Type of anastomosis n (%)	E-S	129 (73.7)	46 (26.3)	*0.036
	E-E	19 (55.9)	15 (44.1)	
Central vein catheter insertion n (%)	Yes	60 (67.4)	29 (32.6)	0.352
	No	88 (73.3)	32 (26.7)	
Type of fistula about the time of creation n (%)	After starting HD	76 (70.4)	32 (29.6)	0.884
	Before starting HD	72 (71.3)	29 (28.7)	
Doppler n (%)	Yes	75 (71.4)	30 (28.6)	0.884
	No	73 (70.2)	31 (29.8)	
Initial (a) function of AVF, n (%)	Yes	131 (92.3)	11 (7.7)	*0.001
	No	17 (25.4)	50 (74.6)	

AVF-arteriovenous fistula; SD-Std. deviation; ESR-erythrocyte; ESR- erythrocyte sedimentation rate; RBC-red blood cell; WBC-white blood cell count; HGB-hemoglobin; PLT-platelet count; Alb-albumin; Glu-glucose; Ca-calcium; PO_4-phosphate; Trig-triglycerides; HDL-high density lipoprotein; LDL-low density lipoprotein; AVF-arteriovenous fistula; E-S-termino-lateral; E-E-termino-terminal; HD-hemodialysis; *statistically significant value

Table 2. *Results of a univariable regression analysis*

Variables	B	p	Exp(B)	95% C.I.for EXP(B)	
				Lower	Upper
Gender	0.460	0.149	1.584	0.848	2.957
Age	-0.005	0.716	0.995	0.968	1.023
ESR	-0.001	0.769	0.999	0.989	1.008
RBC	0.431	0.202	1.539	0.793	2.985
WBC	-0.048	0.357	0.953	0.861	1.056
HGB	0.001	0.912	1.001	0.980	1.023
PLT	0.002	0.235	1.002	0.999	1.006
Alb	-0.033	0.164	0.968	0.924	1.013
Glu	-0.023	0.618	0.977	0.893	1.070
Uric acid	0.001	0.679	1.001	0.998	1.003
Ca	0.074	0.907	1.076	0.315	3.672
PO_4	-0.368	0.240	0.692	0.375	1.279
Systolic blood pressure	-0.005	0.450	0.995	0.984	1.007
Diastolic blood pressure	-0.017	0.131	0.984	0.963	1.005
MEAN blood pressure	0.004	0.685	1.004	0.987	1.021
Cholesterol	-0.037	0.663	0.964	0.815	1.139
Trig	0.018	0.905	1.018	0.763	1.358
HDL	0.701	0.288	2.016	0.553	7.351
LDL	0.003	0.984	1.003	0.778	1.292
Fibrinogen	-0.061	0.545	0.941	0.771	1.147
Intraoperative vein diameter	-0.038	0.947	0.963	0.314	2.949
Intraoperative artery diameter	-0.488	0.297	0.614	0.245	1.535
Location of AVF	-0.893	*0.004	0.409	0.222	.756
Type of anastomosis	0.795	*0.039	2.214	1.040	4.715
Central vein catheter insertion	-0.285	0.353	0.752	0.413	1.371
Type of fistula in relation to the time of creation	-0.044	0.884	0.957	0.527	1.738
Doppler	0.060	0.844	1.062	0.585	1.928
Initial (a)function AVF	3.556	0.001	35.027	15.343	79.962

Reference variable: Functional AVF; AVF-arteriovenous fistula; SD-Std. Deviation; ESR-erythrocyte; ESR-erythrocyte; RBC-red blood cell; WBC-white blood cell count; HGB-hemoglobin; PLT-platelet count; Alb-albumin; Glu-glucose; Ca-calcium; PO_4-phosphate; Trig-triglycerides; HDL-high density lipoprotein; LDL-low density lipoprotein; HD-hemodialysis; *statistically significant value

Table 3. *Results of a multivariable regression models*

Variables in the equation	B	P value	Exp(B)	95% C.I.for EXP(B)	
				Lower	Upper
Location of AVF (radiocephalic vs. brachiocephalic)	1.194	*0.021	3.301	1.197	9.104
Type of anastomosis (E-S vs. E-E)	-1.760	*0.004	0.172	0.052	0.571
Initial (a)function AVF (primary failure vs. initial function)	4.550	*0.001	94.661	28.892	310.147
Constant	2.429	0.125	11.343		

Reference variable: Functional AVF; AVF arteriovenous fistula; E-S -termino-lateral; E-E-termino-terminal; *statistically significant value

DISCUSSION

Arteriovenous fistulas are still the Achilles heel of modern-day hemodialysis. Beyond any doubt, a long-lasting arteriovenous fistula improves the quality of life and reduces morbidity among patients with end-stage renal disease (9). The definition of arteriovenous fistula maturation is challenging. Arteriovenous fistulas should allow the use of two needles, have adequate blood flow for the specific patient, and have minimal recirculation. The 2019 updated vascular access guidelines suggest that AVF maturation should be based on clinical judgment (10). Diabetes mellitus and its associated atherosclerosis effectively narrow the lumen of blood vessels and reduce the blood flow, thereby limiting utilization as an effective conduit. The effect of diabetes mellitus on arteriovenous fistula maturity rates is debatable. Among nephrologists, there are conflicting views, i.e., there is evidence both for and against the influence of diabetes mellitus on the maturation of arteriovenous fistula for hemodialysis (11, 12).

Due to these contradictions, with this research, we set ourselves the task of determining whether among patients with diabetes mellitus, who need an arteriovenous fistula for hemodialysis, some parameters could predict the success of functioning and maturation of the fistula. We found that the determinants of arteriovenous fistula maturation, most important to better understand and predict arteriovenous fistula maturation in our diabetic patients, were not associated with laboratory variables, but with the characteristics of the fistula itself. The result of our research showed that none of the biochemical analysis variables had statistically significant values, in the correlation of subjects with and without maturation of arteriovenous fistulas for hemodialysis, which could perhaps indicate the uniformity of the studied population.

With regards to the association between gender variation and arteriovenous fistula non-maturation, there have been conflicting results reported in the reference literature. Several studies suggested a significant correlation between female gender and decreased patency rates in arteriovenous fistulas, as well as prolonged maturation time before the fistula can be used adequately to sustain hemodialysis sessions. A combination of female gender and increased age (> 65) is significantly associated with non-maturation when compared to men of the same age group (8), probably because of smaller vessel diameters (13). Conte et al. (14) and Salmela et al. (15) found that diabetic patients had significantly lower patency rates, and also female sex and thrombophilia were associated with decreased primary fistula patency rates. Conversely, Sedlacek et al. (16) reported that diabetes was not associated with arteriovenous fistula maturation, and it did not affect either the prevalence of arteriovenous fistula creation in the diabetic group.

Historically, certain patient characteristics have been associated with poor rates of arteriovenous fistula maturation, particularly in females, the elderly, and patients with diabetes mellitus. Our subjects who had a functional fistula and patients who did not have fistula were of the same age, an average of 60 years, thereby eliminating bias. In our study, there were no age differences between male and female patients. Gender differentiation was on the men's side, namely, more than half of the total number of patients were men, and in the group of respondents who had matured fistula, more than two-thirds of the patients were men. However, there were no differences in gender distribution between the two groups, so the gender distribution did not have a decisive influence on fistula maturation in our investigation.

Primary failure, or initial failure of arteriovenous fistula function occurs within 72 hours of surgery when the fistula either thromboses before use or is unsuitable for use. In this study, we used functional maturation, which was defined as access to efficient dialysis using two needles for 75% or more of dialysis sessions over four weeks (17). The meta-analysis by Rooijens et al. (18) showed a pooled estimated primary failure rate of 15.3%, with a tendency to a higher risk of primary failure and a lower primary patency rate when other parameters are evaluated, such as gender, positioning of the arteriovenous fistula in the upper arm, patients with diabetes, etc.

The results of our research, among patients with diabetes mellitus, determined that the initial afunction of arteriovenous fistulas (32%) is twice as high compared to the rate reported by Rooijens et al. (18). The results of our research, among patients with diabetes mellitus, determined that the initial afunction of arteriovenous fistulas (32%) was as twice as high compared to the rate reported by Rooijens et al. (18). Likewise, our results showed that the primary function of the arteriovenous fistula had

a predictive capacity for maturation, which supports the necessity of planning the start of hemodialysis with a mature fistula.

The highest arteriovenous fistula patency was observed in patients with brachiocephalic arteriovenous fistula. These findings may be attributed to the difference in the diameter of the inflow artery (19). Farrington et al. (20) importantly underscored that vascular diameters demonstrated a linear association with arteriovenous fistula maturation with no clear threshold values. The increasingly common choice of creating arteriovenous fistulas with the larger arteries of the upper arm is in line with these findings. Based on the results of 265 arteriovenous fistulas created in patients with diabetes mellitus, Janeckova et al. concluded that initial arteriovenous fistula surgery at the elbow may be a better option than that at the wrist (21).

Intraoperatively measured lumens in our subjects did not significantly affect the rate of fistula maturation, and our study groups did not differ significantly in this variable. On the other hand, the majority of our subjects had brachiocephalic fistulas, which were statistically significantly different from patients with radiocephalic fistulas. What is particularly important for our research is that brachiocephalic fistula has predictive significance for functioning and maturation. Thus, it seems that the diameter of blood vessels used for anastomosis is more important during maturation and that diabetes has an additive role in determining fistula patency.

Surgical technique is of paramount importance for long-term patency of arteriovenous fistula. There are two commonly known techniques to carry out the anastomosis between the veins to the artery: end to side and side to side. Some studies concluded that the side-to-side configuration showed early maturation and higher cumulative patency rates (22). A generally acceptable attitude is that an end-to-side anastomosis is the most recommended, while the end-to-end connection is only used for the reconstruction or primary but emergency access (23). End-to-end anastomosis yields higher rates of hand ischemia, especially in diabetic and elderly patients. Yet, it is currently advised that the method of arteriovenous fistula creation should be selected based on the individual patient's vessel anatomy (24).

Regardless of the recommendations, opinions, or findings in the literature, we had had the experience with the use of end-to-side anastomoses in the majority of our subjects, especially due to the morphological characteristics of the artery walls, which, in most cases, are atherosclerotically altered. This type of anastomosis, in our subjects, showed statistically significant differences between patients with and without functional maturation of the fistula. Moreover, end-to-side anastomosis has shown its importance in the prediction of fistula maturation.

Limitation

As this was a retrospective analysis of our cohort, we were unable to actively recruit further patients to increase the sample size, which can make it difficult to generalize the conclusions to the entire population, although we had relatively uniform results when it comes to the demographic and gender characteristics of the respondents. We tried to stratify the sample as far as this type of study allowed, but were not sure that all the confounding factors were eliminated. Likewise, unfortunately, we did not have information on the type of diabetes mellitus, which, again, could affect the achieved results.

CONCLUSION

Predictors of maturation of arteriovenous fistula for hemodialysis in our study, among the population of patients with diabetes mellitus, are initial fistula function, brachiocephalic location, and terminolateral type of anastomosis.

Future studies, with a well-stratified sample, should demonstrate the influence of metabolic parameters on arteriovenous fistula maturation.

Conflict of interest

The authors declared no conflicts of interest concerning the authorship and/or publication of this article.

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Prediktivni parametri maturacije arteriovenske fistule za hemodijalizu kod bolesnika sa dijabetesom melitusom

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SAŽETAK

Uvod/Cilj. Arteriovenska fistula (AVF) preporučuje se kao idealan vaskularni pristup za hemodijalizu (HD). Međutim, postoje oprečna mišljenja kada su posredi bolesnici sa dijabetesom melitusom.
Cilj ove studije bilo je određivanje prediktivnih parametara sazrevanja AVF-a za hemodijalizu kod bolesnika sa dijabetesom melitusom.
Metodologija. Istraživanje je organizovano kao retrospektivna, deskriptivno-analitička studija. Ciljnu grupu našeg istraživanja činilo je 209 bolesnika sa dijabetesom melitusom, kojima je kreiran AVF za hemodijalizu. Evidentirali smo demografske i polne karakteristike, lokaciju i tip AVF-a u odnosu na vreme kreiranja, vrstu anastomoze, podatke o početnoj funkciji, odnosno odsustvu funkcije fistule, postavljanju katetera za hemodijalizu i vrednostima krvnog pritiska. Pre operacije urađen je dopler ultrazvuk krvnih sudova, a intraoperativno su mereni lumen arterije i vene koje su korišćene za kreiranje AVF-a. Analizirali smo laboratorijske varijable koje se rutinski kontrolišu u našoj ustanovi.
Rezultati. Osobe sa dijabetesom sa uspešno sazrelim fistulama značajno češće imale su proksimalno lociran AVF (p = 0,004), termino-lateralni tip anastomoze (p = 0,036) i inicijalnu funkciju (p = 0,001). U univarijantnoj analizi, brahiocefalični AVF (p = 0,004), termino-lateralni tip anastomoze (p = 0,039) i početna funkcija AVF-a (p = 0,001) bili su prediktivni parametri sazrevanja AVF-a. Multivarijantna statistička analiza pokazala je da su brahiocefalna lokalizacija AVF-a (p = 0,021), termino-lateralna anastomoza (p = 0,004) i inicijalna funkcija AVF-a (p = 0,001) prediktivni parametri sazrevanja AVF-a kod osoba sa dijabetesom.
Zaključak. Kao prediktivni parametri sazrevanja fistule kod bolesnika sa dijabetesom melitusom u našoj studiji izdvojili su se početna funkcija AVF-a, brahiocefalične lokacije i termino-lateralni tip anastomoze.

Ključne reči: **arteriovenska fistula, hemodijaliza, dijabetes melitus, sazrevanje, prediktivni parametri**

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Original article

Could De Ritis Score Be a Useful Predictor of Mortality in COVID-19 Patients Who Require Intensive Care?

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SUMMARY

Introduction/Aim. Inflammatory markers are being investigated as possible predictors of mortality in intensive care population. COVID-19 infection causes significant amount of inflammatory burden. De Ritis score has been suggested as a novel disease marker in conditions characterized with inflammation. In the present work, we aimed to compare De Ritis scores of deceased and survived COVID-19 patients in an institutional intensive care unit.
Methods. Patients treated in intensive care unit with a diagnosis of COVID-19 infection were enrolled in the study. De Ritis scores of the deceased and survived subjects were compared.
Results. The De Ritis score among survivors and non-survivors was 1.12 (range: 0.3–6.9)% and 1.43 (range: 0.2–16)%, respectively, with a statistically significant difference ($p = 0.03$). Additionally, the De Ritis score exhibited significant positive correlations with lactate dehydrogenase (LDH) ($r = 0.37$, $p < 0.001$), D-dimer ($r = 0.38$, $p < 0.001$), and C-reactive protein (CRP) ($r = 0.19$, $p = 0.01$) levels. When the De Ritis score exceeded the 1.32% threshold, its sensitivity and specificity in predicting mortality were 60% and 61%, respectively, with an area under the curve (AUC) of 0.61 ($p = 0.03$, 95% confidence interval: 0.52–0.7). Furthermore, each unit increase in the De Ritis score was associated with a 96% increase in the odds of mortality among COVID-19 patients treated in the intensive care unit ($p = 0.03$, OR: 0.96, 95% confidence interval: 0.86–0.98).
Conclusion. De Ritis score can be a useful marker of poor prognosis in COVID-19 patients in intensive care units.

Keywords: **De Ritis score, inflammation, COVID-19, intensive care**

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INTRODUCTION

Intensive care unit (ICU) serves as critical hubs for patients battling severe illnesses and life-threatening conditions. Within these high-stakes medical environments, one of the paramount concerns is mortality, as healthcare providers strive to improve patient outcomes. In recent years, there has been a growing recognition of the intricate relationship between mortality in the ICU and the use of inflammatory markers as valuable prognostic tools. Inflammation, a complex biological response triggered by the body in response to injury or infection, plays a central role in many critical illnesses. Consequently, monitoring inflammatory markers in ICU patients has emerged as a promising avenue for predicting outcomes, tailoring treatment strategies, and enhancing overall patient care. Recent research has uncovered a notable link between mortality among patients in the intensive care unit and elevated levels of inflammatory markers in the bloodstream. These markers encompass mean platelet volume (1), the ratio of C-reactive protein to albumin (2), and the systemic inflammatory index (3).

COVID-19 infection typically initiates with flu-like symptoms (4) and can manifest as asymptomatic or range from mild to severe in severity (5). The infection is characterized by a significant inflammatory burden (6). Research has investigated the association between hemogram parameters and COVID-19 infection, revealing that neutrophil to lymphocyte ratio (NLR) (7) and several other inflammation markers (8) are linked to the infection. Additionally, red cell distribution width, an indicator of anisocytosis in the hemogram, has been linked to recurrent hospitalizations in COVID-19 patients (9). Furthermore, certain inflammatory markers have been identified as predictors of frailty in diabetic individuals during COVID-19 (10). Hence, it can be inferred that inflammatory indices may be correlated with COVID-19 infection.

The De Ritis score, named after the Italian physician Fernando De Ritis, is a widely used diagnostic tool in the field of clinical medicine (11). This simple yet informative score is primarily employed to assess liver function and help diagnose various liver-related disorders. By examining the ratio of two essential enzymes in the bloodstream, the aspartate aminotransferase (AST) and alanine aminotransferase (ALT), the De Ritis score offers valuable insights into the health and functioning of the liver. In this introduction, we will delve into the significance of the De Ritis score, its calculation, and its crucial role in aiding medical professionals in diagnosing and monitoring liver diseases. It has been linked to inflammatory conditions i.e., thyroiditis (12). Moreover, it has been linked with renal and respiratory dysfunction (13) and mortality (14).

In this retrospective study, our objective was to compare the De Ritis scores of intensive care patients who survived with those of deceased subjects.

PATIENTS AND METHODS

Following the approval of the study protocol by the local Ethics Committee (approval date: August 22, 2023, approval number: 2023/274), we included COVID-19 patients from the intensive care unit in the current retrospective study. We analyzed the data of the patients treated in our institution between January 2021 till December 2021. The study group was divided according to the outcome—into survived and deceased patients' groups. Subjects younger than 18 years of age, with established acute/chronic liver disease, and pregnant women were excluded from the study. Age, gender, and the presence of comorbidities such as diabetes mellitus, hypertension, cancer, cardiovascular diseases, chronic obstructive pulmonary disease, and chronic kidney disease were recorded after retrieving data from patients' files and the institutional database. Laboratory parameters of the participants, including leukocyte count (WBC), hemoglobin (Hb), platelet count (PLT), blood urea, serum creatinine, plasma glucose, aspartate and alanine transaminases (AST, ALT), serum albumin, lactate dehydrogenase (LDH), D-dimer, ferritin, and C-reactive protein (CRP) values of the study population were noted. The De Ritis score is derived by dividing AST by ALT. Subsequently, data from both survived and deceased subjects were compared.

Statistics

Statistical analyses were performed using SPSS 18.0 for Windows (IBM Co., Armonk, NY, USA). The homogeneity of study variables was assessed using the Kolmogorov-Smirnov test. Continuous variables conforming to a normal distribution were presented as means and standard deviations and compared using independent t-test samples. Continuous variables not adhering to a normal dis-

tribution were presented as medians (range) and compared using the Mann-Whitney U test. Categorical variables were compared using the Chi-square test and expressed as percentages. Pearson's correlation analysis was conducted to explore potential correlations among study variables. Receiver operating characteristic (ROC) curve analysis was employed to determine the sensitivity and specificity of the De Ritis score in predicting mortality. Binary logistic regression analysis was utilized to ascertain whether the De Ritis score independently constituted a risk factor for mortality in the ICU population. Statistical significance was defined as a probability (p) value greater than 0.05.

RESULTS

Among the patients, there were 133 deceased individuals and 146 survivors. The mean ages of the survived and deceased subjects were 75 years (± 13) and 65 years (± 17), respectively ($p < 0.001$). Respectively, 59% and 63% of the survived and deceased patients were men ($p = 0.63$). Diabetes mellitus was found in 20% and 37% of the survived and deceased COVID-19 patients, respectively ($p = 0.03$). The rates of hypertension (65% in survivors versus 72% in deceased; $p = 0.41$), cancer (7% in survivors versus 14% in deceased; $p = 0.17$), cardiovascular diseases (30% in survivors versus 35% in deceased; $p = 0.57$), chronic obstructive pulmonary disease (11% in survivors versus 22% in deceased group; $p = 0.11$), and chronic kidney disease (7% in survivors versus 10% in deceased; $p = 0.43$) were similar in survived and deceased patients.

Median De Ritis score of the survived and deceased subjects were 1.12 (0.3–6.9)% and 1.43 (0.2–16)%, respectively. De Ritis score was significantly higher in deceased group compared to the survived group ($p = 0.03$). Data of the survived and deceased groups were summarized in Table 1.

The De Ritis score exhibited significant positive correlations with LDH ($r = 0.37$, $p < 0.001$), D-dimer ($r = 0.38$, $p < 0.001$), and CRP ($r = 0.19$, $p = 0.01$) levels.

In the ROC analysis, when the De Ritis score exceeded the 1.32% threshold, it demonstrated a sensitivity of 60% and specificity of 61% in predicting mortality, with an AUC of 0.61 ($p = 0.03$, 95% confidence interval: 0.52-0.7). Figure 1 illustrates the ROC curve of the De Ritis score for mortality detection.

Table 1. *Data of the deceased and survived COVID-19 patients in intensive care unit*

	Deceased	**Survived**	p
	Mean ± SD		
Age (years)	75 ± 13	65 ± 17	**< 0.001**
Hb (Hb)	13.4 ± 1.3	13.3 ± 1.5	0.64
Serum albumin (g/dL)	2.8 ± 0.5	3.2 ± 0.6	**0.001**
	Median (min-max)		
WBC (k/mm^3)	12.4 (0.5-48)	11 (2.8-36)	0.13
PLT (k/mm^3)	187 (27-618)	230 (29-505)	**0.02**
LDH (U/L)	524 (230-1500)	449 (219-1041)	**0.03**
D-dimer (mg/L)	3 (0.3-72)	3 (0.2-75)	0.3
Ferritin (ng/mL)	789 (21-2000)	490 (19-2000)	**0.004**
CRP (mg/L)	112 (0.5-350)	68 (0.1-344)	**0.003**
Urea (mg/dL)	71 (17-396)	47 (17-186)	**< 0.001**
Creatinine (mg/dL)	1.2 (0.4-11.4)	0.9 (0.4-8.3)	**0.001**
Glucose (mg/dL)	148 (69-618)	133 (75-415)	0.12
AST (U/L)	46 (7-2730)	38 (13-428)	**0.03**
ALT (U/L)	34 (6-1344)	39 (8-87)	0.87
De Ritis score (%)	1.43 (0.2 -16)	1.12 (0.3-6.9)	**0.03**

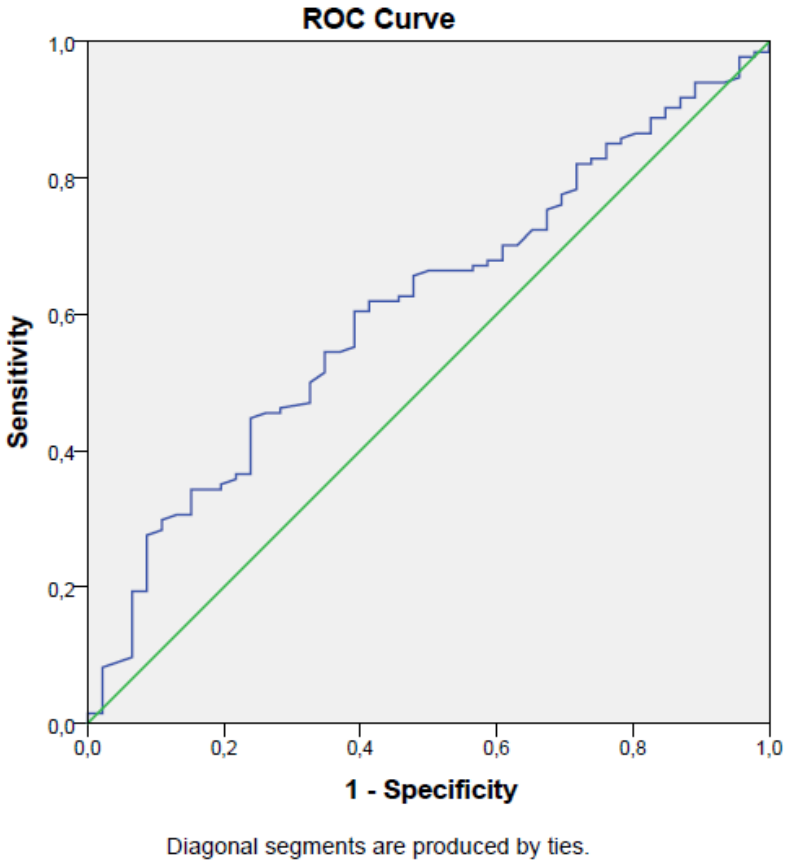

Figure 1. *ROC curve of De Ritis score in detecting mortality in ICU COVID-19 patients*

In binary logistic regression analysis, after adjustment of age, lactate dehydrogenase (LDH), ferritin, CRP and D-dimer, De Ritis score was found to be an independent risk factor for mortality in ICU population (p = 0.03, OR: 0.96, 95% confidence interval: 0.86-0.98). An increment of one unit in the De Ritis score elevated the odds of mortality by 96% among COVID-19 patients undergoing treatment in the intensive care unit.

DISCUSSION

The present study showed that De Ritis score could be a useful marker of mortality in ICU population with COVID-19 infection, since it is significantly increased in deceased subjects compared to the survivors. Furthermore, the De Ritis score showed a significant correlation with other inflammation markers such as CRP, LDH, and D-dimer. Additionally, notable sensitivity and specificity were observed in using the De Ritis score to predict mortality. Finally, regression analyses revealed that De Ritis score was an independent risk factor of mortality in ICU patients.

Inflammation is associated with COVID-19 infection (6). Higher burden of inflammation is noted in subjects with more serious disease course. Moreover, advanced elevation in inflammatory markers was noted in COVID-19 patients with poor outcome (15). De Ritis score is also elevated in conditions characterized with inflammation. Several examples may include malignant diseases (16), viral infections (17), sepsis (18), cerebrovascular diseases (19), and cardiac conditions (14). Given the association between COVID-19 infection and inflammation, the higher De Ritis scores observed in deceased patients compared to survivors in the present study align with existing literature findings.

Albitar et al. studied mortality risk factors in COVID-19 patients and found that male gender, older age, presence of diabetes mellitus, and hypertension independently increased the risk of mortality

in this population (20). Moreover, a meta-analysis revealed similar results by reporting advanced age, male gender, and accompanied chronic diseases as factors associated with mortality (21). Having diabetes mellitus was indeed an important factor that was associated with mortality in COVID-19 patients, according to the study by Shi et al. (22). Besides advanced age and male sex, a number of clinical factors including impaired kidney function, hypotension, tachypnea, hypoxia, elevated D-dimer, and elevated troponin were reported to be associated with COVID-19 mortality (23). Similarly, we found that deceased subjects were older, had higher serum creatinine levels and more frequently had the associated diabetes mellitus. Nevertheless, in our study, there were no significant differences in gender and D-dimer levels between deceased and survived patients. Additionally, comorbidities such as hypertension, cancer, cardiovascular diseases, chronic obstructive pulmonary disease, and chronic kidney disease were present in both the survived and deceased groups at similar rates, with no significant variation.

In the current study, serum CRP levels were elevated in the deceased subjects compared to the survivors. There are similar reports in the literature. High levels of CRP were reported in severe COVID-19 infection (24). Progression and severity of COVID-19 infection are well predicted with serum CRP levels, according to the Yitbarek et al's study (25). Subsequently, their findings were confirmed by another report (26). These data suggest that CRP was associated with severe cases of COVID-19. Our data further showed that CRP was associated with mortality in this population.

We observed higher LDH and ferritin levels in deceased COVID-19 patients when compared to those who survived. Poor prognosis was noted in a meta-analysis (27) in COVID-19 cases with elevated LDH levels. Furthermore, increased LDH was suggested as an independent risk factor for mortality in COVID-19 infection (28). Similarly, authors found serum ferritin as a reliable marker of inflammation and disease severity in COVID-19 patients (29, 30). These data are confirmed by our report and we further revealed that De Ritis score was correlated with CRP, LDH and D-dimer levels in COVID-19 population.

There are several limitations of the present work. First, retrospective design allows us only to clarify a simple association rather than causal relationship between COVID-19 mortality and De Ritis score. Second, single center nature of our study makes it difficult to globalize the study results. Third, a relatively small study population could be another limitation. However, this is one of the first studies reporting the association between De Ritis score and COVID-19 mortality in patients who required management in intensive care unit.

CONCLUSION

In conclusion, we find that the De Ritis score could make a useful, inexpensive, and easy to assess tool in determining the mortality risk of COVID-19 patients.

Authorship

IK and KII participated in the conception and design of the study, data collection and analysis, interpretation of the findings, drafting of the manuscript, and critical revisions for important intellectual content, and provided the final approval. BO and GA contributed to the conception and design of the study, analysis and interpretation of the data, drafting of the manuscript, critical revisions for important intellectual content, and provided the final approval. DRS contributed to critical statistical revisions of the manuscript for important intellectual content and provided the final approval.

Data availability statement

The data related to this work is available by the corresponding author upon reasonable requests.

Conflict of Interest

None to declare.

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Da li bi De Ritisov skor mogao biti koristan prediktor mortaliteta kod bolesnika sa kovidom 19 na intenzivnoj nezi?

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SAŽETAK

Uvod/Cilj. Markeri inflamacije istražuju se kao mogući prediktori mortaliteta kod bolesnika na intenzivnoj nezi. Infekcija kovidom 19 predstavlja značajan uzrok inflamacija. De Ritisov skor je predložen kao nov marker bolesti u uslovima karakterističnim za upalu. Cilj ovog rada bilo je upoređivanje De Ritisovih skorova kod bolesnika preminulih nakon komplikacija izazvanih kovidom 19 i bolesnika koji su preživeli infekciju kovidom 19 u institucionalnoj jedinici intenzivne nege.
Metode. Studija je obuhvatila bolesnike lečene u jedinici intenzivne nege sa dijagnozom infekcije kovidom 19. Upoređivani su rezultati De Ritisovog skora bolesnika koji su preminuli i bolesnika koji su preživeli.
Rezultati. De Ritisov skor među preživelim i preminulim bolesnicima bio je 1,12 (opseg: 0,3%–6,9%) i 1,43 (opseg: 0,2%–16%), redom, sa statistički značajnom razlikom (p = 0,03). Pored toga, De Ritisov skor pokazao je značajne pozitivne korelacije sa nivoima laktat dehidrogenaze (LDH) (r = 0,37, p < 0,001), D-dimera (r = 0,38, p < 0,001) i C-reaktivnog proteina (CRP) (r = 0,19, p = 0,01). Kada je De Ritisov skor premašio prag od 1,32%, njegova osetljivost i specifičnost u predviđanju smrtnosti bili su 60% i 61% za bolesnike koji su preminuli i one koji su preživeli, redom, sa površinom ispod krive (engl. *area under curve* – AUC) od 0,61 (p = 0,03, 95% interval pouzdanosti: 0,52–0,7). Štaviše, svako povećanje jedinice u De Ritisovom skoru bilo je povezano sa povećanjem šanse za smrtnost od 96% među bolesnicima sa kovidom 19 lečenim na odeljenju intenzivne nege (p = 0,03, OR: 0,96, 95% interval pouzdanosti: 0,86–0,98).
Zaključak. De Ritisov skor može biti koristan marker loše prognoze kod bolesnika sa kovidom 19 u jedinicama intenzivne nege.

Ključne reči: **De Ritisov skor, inflamacija, kovid 19, intenzivna nega**

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Original article

Quality of Sleep in Third-Trimester Pregnancy and Non-Psychotic Postpartum Depression

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SUMMARY

Introduction/Aim. Quality of sleep (QoS) in the third-trimester of pregnancy has been suggested as a potential risk factor for the onset of non-psychotic postpartum depression (NPPD). The aim of the paper was to investigate whether the quality of sleep in the third-trimester of pregnancy is a risk factor for non-psychotic postpartum depression (NPPD) in women without a diagnosed psychiatric disorder.
Method. In the third-trimester of pregnancy, 218 pregnant women completed a questionnaire constructed for research purposes as well as the Pittsburgh Sleep Quality Index (PSQI). Four weeks after childbirth, the participants filled in the Edinburgh Postnatal Depression Scale (EPDS) and received structured interview diagnoses.
Results. High risk for NPPD (score on EPDS ≥ 13) was found in 21 (9.63%) participants. Higher rates on the EPDS were noticed in single, unemployed, housewives, women who were getting little social support, women who were dissatisfied with their annual household income and with unwanted pregnancy ($p < 0.05$). Social support and QoS were positively related to NPPD, whereas annual household income and marital satisfaction were negatively related to NPPD. The third-trimester QoS was related to NPPD symptoms, and the correlation was statistically significant ($p < 0.05$).
Conclusion. Poor third-trimester QoS is a risk factor for NPPD.

Keywords: **quality of sleep, non-psychotic postpartum depression, risk factors**

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INTRODUCTION

Motherhood is considered the most beautiful but also a potentially stressful event, which, in addition to changes on the somatic level, causes some significant changes in psychological and social functioning, especially in the domain of sleep. Throughout pregnancy, and especially in the third-trimester of pregnancy, women face problems with timing, reduced sleep duration, and maintaining good quality of sleep (QoS) (1).

Postpartum is a particularly vulnerable period for the development of non-psychotic postpartum depression (NPPD) in the first four weeks after childbirth (2). A current comprehensive literature review on the global epidemiology of NPPD, which in the final analysis included a total of 565 studies from 80 different countries or regions, gives an overall prevalence of 17.22% (3). NPPD has been shown to have an impact on attachment, behavioral and cognitive development of the growing baby, the relationship with the partner, and the functionality of the family as a whole (2, 4).

Up to date, the factors that have been associated with the occurrence of NPPD are difficult and prolonged childbirth accompanied with complications, subjective experience of lack of social support, fear of abandonment, unplanned/unwanted pregnancy, maternal age, level of education, and depression, anxiety and stress during pregnancy (2, 5). Poor QoS in the third-trimester of pregnancy has been suggested as a potential risk factor for the onset of NPPD, in the antepartum depressed women (6). Insufficient and disturbed sleep affects memory, decision-making, as well as mood during and after pregnancy (6). Longer-term sleep disorders can represent a prodrome, but also a risk factor for the later development of NPPD, allowing the possibility of early intervention (1, 6).

The aim of the paper was to investigate whether the poor quality of sleep in the third-trimester of pregnancy is a risk factor for NPPD, in a sample without a diagnosed psychiatric disorder.

METHOD

Study subjects

The research was designed as a prospective study with two observation periods, in the third-trimester of pregnancy and the fourth week postpartum. From January 2019 to June 2019, 236 pregnant women who attended the Parenting school at the Department of Gynecology and Obstetrics in Primary Health Center Niš were invited to participate in the research. Of the total number, six women initially refused to participate for private reasons, and 18 women did not meet the inclusion criteria.

Participants with previously and/or during pregnancy detected psychiatric disorders, application of drugs of any type and any detected somatic process, which could potentially be an etiological factor and explain the presented symptomatology (primarily endocrinological, inflammatory, or autoimmune process) were excluded. We advised them to avoid the use of tea or coffee inasmuch not to affect the quality of sleep.

Pregnant women who were excluded did not differ significantly in terms of age, marital status, unplanned pregnancy, or unemployment, nor did they have a lower level of education compared to the observed sample, so it was unlikely that they were exposed to a greater risk than the participants in the research. Also, the rate of refusal to participate in the study was relatively low, which suggests that our study was representative of women who managed their pregnancy in the given period in the Primary Health Center in Niš. Two hundred and eighteen pregnant women gave their verbal and signed informed consent after a detailed explanation of the research concept.

In the third-trimester of pregnancy, they were invited by trained staff to complete a questionnaire constructed for research purposes (which included questions related to sociodemographic, health and pregnancy data) and Standard Pittsburgh Sleep Quality Index (PSQI). Four weeks after childbirth, the participants filled in the Edinburgh Postnatal Depression Scale (EPDS) and received structured interview diagnoses. The data obtained from the participants were additionally confirmed by examining the medical documentation. They were also explained that participation was fully on voluntary basis.

In the group of test participants, a psychiatric diagnosis was established by a specialist psychiatrist, based on the diagnostic criteria of the available classification systems, confirmed by the use of the Serbian version of the Mini International Neuropsychiatric Interview, version 6.0, which is used both in clinical and research work (7-11).

The design of the study was approved by the Ethics Committee of the Faculty of Medicine of the University of Niš and the Ethics Committee of the Health Center in Niš. All research procedures carried out in the course of this research were under the ethical standards of the Declaration of Helsinki from 1975, revised in 2013, Basics of Good Clinical Practice and the Law on Health Care of the Republic of Serbia.

Research scales

Sociodemographic and pregnancy-related questions included their age, educational level, satisfaction with perseived social support, marital satisfaction, annual household income, employment status, partners' employment status, place of residence, wanted or unwanted pregnancy, number of children, the age of their youngest child, sex of the child, and type of delivery. Participants were asked to indicate their diagnosed illnesses and any history of psychiatric disorders, such as NPPD. Finally, the participants were asked whether they had any complications with this pregnancy, and whether they were physically active, consumed alcohol or smoked.

The PSQI quality of sleep questionnaire consists of 19 self-rated questions, i.e., nine questions, and the fifth question contains 10 items (12). These 19 self-rated questions are classified into seven groups, each scores from 0-3, and the total score scale varies from 0 to 21. A total score of 5 or greater is indicative of the poor quality of sleep, and a higher score indicates the worse quality of sleep. It assesses a one-month interval and provides data useful both in clinical and scientific work, as it measures the QoS, duration and latency of sleep, common efficacy of sleep, and functionality during the day. PSQI is a widely used instrument for subjective QoS assessment which is translated and standardized in Serbia (13-15). The Serbian version of the scale has good reliability and validity (15). PSQI is understandable to the examinees and applicable in everyday clinical practice.

EPDS is a generally accepted and widely used assessment scale for postpartum depression (16). It is a reliable and valid screening method for detecting symptoms in the postpartum period, which indicate the risk of developing postpartum depression, tested on different populations, validated in Serbia (17-21). It is a 10 item self report scale, testing: mood, contentment, feeling guilty, anxiety, dread, insomnia, resourcefulness, grief, tearfulness, and self-injury. Each item is divided into 0–3 points and the total score ranges from 0 to 30 points. The final score greater than 10 is a risk of postpartum depression, and those who scored ≥ 13 were considered as NPPD women.

Statistical analysis

All data were statistically processed with IBM SPSS statistical software (version 21) for the Windows operating system. Numerical data were presented as mean ± standard deviation. Sociodemographic characteristics data were evaluated by their number and percentage of dispersion. Statistical description methods calculated mean values, standard deviations, frequencies, prevalence rates, and confidence intervals. For initial analysis, a Student's t test was used to determine statistical differences in continuous variables between the groups. A Chi-square tested difference in the prevalence rates of postpartum depression among different socio-demographic factors. The correlation between sociodemographic factors, third-trimester sleep quality, and postpartum depression was analyzed by using the binary classification non-conditional logistic regression model test. Linear regression method was used to analyze the correlation between third-trimester sleep quality and postpartum depressive symptoms. Results were evaluated at a 95% confidence interval, and significance was evaluated at $p < 0.05$.

RESULTS

Sociodemographic characteristics and NPPD prevalence rate

Participants with a high risk for NPPD had the score on EPDS ≥ 13 (Figure 1). When filling out the assigned questionnaires for the first time, the average gestational age was 31.8 ± 1.6 weeks (range 32–36 weeks). Sociodemographic features of participants are presented in Table 1.

Statistically significant correlations between EPDS scores and additional characteristics of the participants are presented in Table 2. No statistically significant influence was noted in terms of their partners' employment status, or place of residence. Also, we did not find a statistically significant correlation between scores obtained on EPDS with type of the delivery, number of children, the age of their youngest child, and sex of the child.

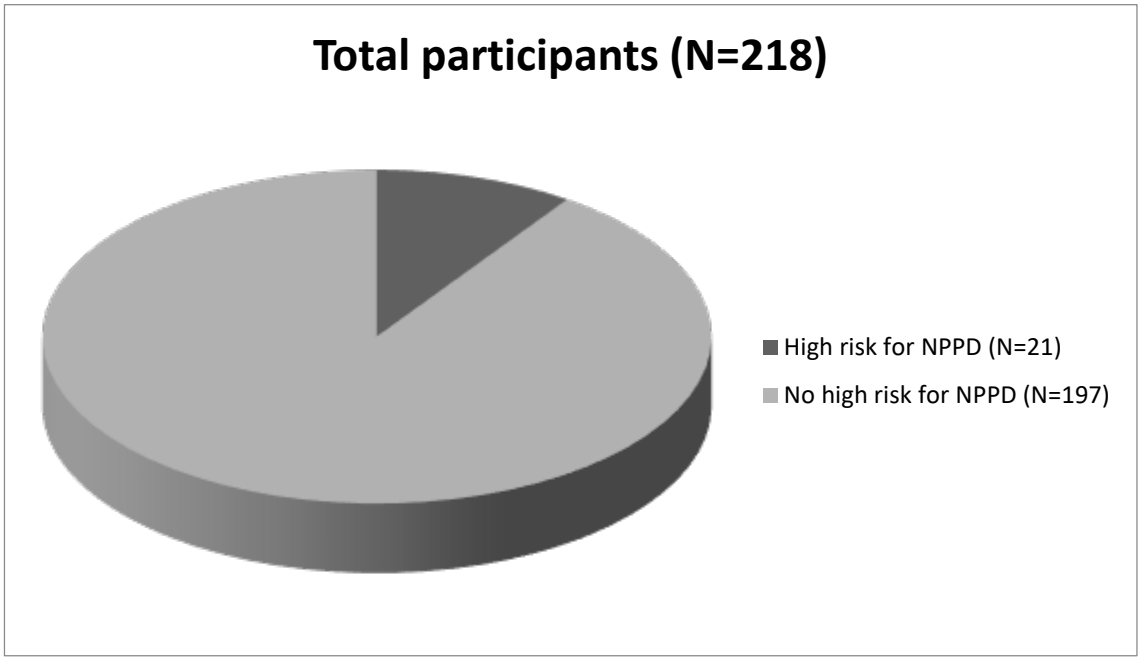

Figure1. *Participants with and without high risk for NPPD*

Table1. *Sociodemographic characteristics of participants (N = 218)*

Feature	Value
Gestational age (mean ± SD, median (min - max), weeks)	31.8 ± 1.6; (32-36)
Faculty and above (%)	53.22
High school education or below (%)	46.79
Took work and maternity leave (%)	36.69
Had no job or housewives (%)	63.31
Low income (%)	33.48
Middle income (%)	24.77
Higher income (%)	41.75

Table 2. *Correlation between EPDS scores and additional characteristics of participants*

		Single	Unemployed and housewives	Little social support	Dissatisfied with incomes	Unwanted pregnancy
EPDS scores	R_p	0.142	0.189	0.193	0.186	0.202
	p	< 0.05*	< 0.05*	< 0.05*	< 0.05*	< 0.05*

p < 0.05*

Third-trimester quality of sleep, demographic characteristics, and NPPD

The results demonstrated that emotional status, employment status, social support, annual household income, marital satisfaction and QoS were significantly related to NPPD; among these, social support and QoS were positively linked to NPPD, and annual household income and marital satisfaction were negatively related to NPPD. Third-trimester QoS was linked to NPPD symptoms, and the correlation was statistically significant. Poorer QoS was associated with higher score on EPDS. As a causal relationship was observed between QoS in the third-trimester of pregnancy and a high score on the EPDS (≥ 13), QoS could be considered as a prospective risk factor for the NPPD.

DISCUSSION

Our findings showed that within four weeks after delivery, the prevalence of NPPD was 9.63% in a sample without antepartum mood disorder, which is a relatively similar value obtained in research on this population in Serbia, however, it is lower than the values reported by meta-analyses (3, 17, 20, 22). NPPD in our research was significantly associated to emotional and employment status, social support, annual household income, marital satisfaction, and QoS.

In our study, we found the causal correlation between poor QoS in the third-trimester of pregnancy and NPPD, in a sample without diagnosed any psychiatric disorder, particularly antepartum depression. Studies have associated so far sleep disorders, particularly insomnia in the third trimester of pregnancy with mood disorders during pregnancy and postpartum, due to the changes in physiologic sleep-wake cycle and more cortical arousal (1, 22, 23). In addition, research conducted on a group of pregnant and currently non-depressed women, but with a history of mood disorders, found a connection between the QoS in late pregnancy and the recurrence of a depressive episode (1).

Poor QoS is so far associated with several sociodemographic factors in Serbia, but according to the data available to us, it has not been observed as a risk factor or investigated in the population of pregnant and postpartum women (13, 14). The latest data from the field of sleep disorders indicate that especially the QoS is important for the mental health of women in the first months after childbirth, as it could have a negative impact on the severity of depressive symptoms in NPPD women, including the risk of suicide (24). In a study of non-depressed postpartum women, results on polysomnography found increased deep restorative stage and decreased full sleep time, indicating that their sleep was more efficient and suggesting that women with poor QoS are vulnerable to mood disorders (25).

Being single, unsatisfied in marriage, bad economic situation, unemployment and lack of own income had been shown to be significantly contributing risk factors for the occurrence of NPPD in previous research, which our study confirmed (2, 26). Also, higher rates on the EPDS were noticed in women who were getting little social support and with unwanted pregnancy.

The most cited is the correlation between dysfunctional partner relations and the absence of social support and postpartum depression, which explains the Beck's interpersonal model of postpartum depression of the mismatch between expected and desired social support. Satisfaction with perceived and received social support is thought to be a protective factor against the development of NPPD, and studies have linked the lower social support with NPPD, similar to the result of the present study (2, 5, 17).

The importance of this research is great. Namely, according to the data available to us, this is the first prospective research designed in this way in our country, which contributes to better understanding the QoS as an important component of preserving the mental health in this vulnerable population and as a potential risk factor for the NPPD.

Admittedly, the research has several limitations. First, we did not use polysomnography for objective assessment, because we wanted to examine the relationship between subjective QoS assessment and NPPD. Second, the research did not assess cognitive bias before completing the self-assessment questionnaire. The third participation in the research was exclusively on a voluntary basis.

CONCLUSIONS

The results of our research showed a correlation between poor QoS in the third-trimester of pregnancy and several sociodemographic factors and a high score on the EPDS. QoS can be routinely measured in daily clinical work, using the PSQI questionnaire, thus potentially preventing NPPD.

Well-developed longitudinal studies with larger samples are needed for better understanding potential risk factors, severity of NPPD, and early preventive measures.

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Declaration of conflicting interests

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Kvalitet spavanja u trećem tromesečju trudnoće i nepsihotična postporođajna depresija

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S A Ž E T A K

Uvod. Kvalitet spavanja (engl. *quality of sleep* – QoS) u trećem tromesečju trudnoće može se smatrati potencijalnim faktorom rizika za nastanak nepsihotične postporođajne depresije (engl. *non-psychotic postpartum depression* – NPPD).
Cilj. Cilj našeg istraživanja bio je da istražimo da li je kvalitet spavanja u trećem tromesečju trudnoće faktor rizika za nastanak nepsihotične postporođajne depresije kod žena kod kojih nije postavljena dijagnoza psihijatrijskog poremećaja.
Metode. 218 trudnica u trećem tromesečju trudnoće, popunilo je upitnik konstruisan za potrebe istraživanja, kao i Pitsburški indeks kvaliteta spavanja (engl. *Pittsburgh sleep quality index* – PSQI). Četiri nedelje nakon porođaja, učesnice su popunile Edinburšku skalu postnatalne depresije (engl. *Edinburgh postnatal depression scale* – EPDS), a dijagnoza je postavljena primenom strukturisanog intervjua.
Rezultati. Visok rizik od nastanka NPPD-a (skor na EPDS-u ≥ 13) nađen je kod 21 učesnice istraživanja (9,63%). Visok skor na EPDS-u uočen je kod žena bez partnera, nezaposlenih i domaćica, žena sa niskim nivoom socijalne podrške, žena nezadovoljnih godišnjim prihodom u domaćinstvu i kod žena prilikom neželjene trudnoće ($p < 0,05$). Socijalna podrška i kvalitet spavanja bili su u pozitivnoj korelaciji, a godišnji prihod domaćinstva i zadovoljstvo partnerskim odnosom u negativnoj korelaciji sa nepsihotičnom postporođajnom depresijom. Kvalitet spavanja u trećem tromesečju povezan je sa simptomima nepsihotične postporođajne depresije, a korelacija je statistički značajna ($p < 0,05$).
Zaključak. Loš kvalitet spavanja predstavlja faktor rizika za nastanak nepsihotične postporođajne depresije.

Ključne reči: **kvalitet spavanja, nepsihotična postporođajna depresija, faktori rizika**

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Case report

Wernicke Encephalopathy: Late-Stage Symptoms

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SUMMARY

Introduction. Wernicke encephalopathy is a rare acute/subacute neurological disorder, commonly caused by prolonged thiamine deficiency in patients who chronically consume alcohol. According to the Caine classification criteria, the clinical diagnosis of this encephalopathy involves at least two of the following four signs: nutritional deficiency, oculomotor dysfunction, ataxia, and changes in the mental status. This case report highlights rare clinical signs in the late stage of the disease, as well as the consequences of possible local hypoperfusion of the brainstem in the form of an ischemic vascular event.
Case report. A 39-year-old female patient (previously treated at a regional general hospital) was admitted to the Department of Emergency Neurology at the University Clinical Center of Vojvodina with a history of a series of epileptic seizures, altered consciousness, oculomotor signs, opisthotonus, and cognitive dysfunction, following years of alcohol consumption and nutritional deficiency. The diagnosis was confirmed by typical neuroimaging findings and specific laboratory tests. Hypertonia with subsequent opisthotonus was one of the clinical manifestations in our patient, while the occurrence of an ischemic stroke was an unexpected event. Empirical administration of high-dose thiamine, along with additional supportive intensive therapy, did not yield satisfactory outcomes.
Conclusion. Wernicke encephalopathy represents a clinical diagnosis based on physical and neurological examination, with neuroimaging. Early recognition of both common and unusual symptoms, particularly in the late stage of the disease, could potentially reduce morbidity and mortality. It is essential to administer thiamine before glucose infusion to all patients with an undetermined cause of altered consciousness.

Keywords: **thiamine, Wernicke encephalopathy, alcoholism, opisthotonus, stroke**

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INTRODUCTION

Wernicke encephalopathy (WE) is a rare, devastating acute or subacute neurological disorder caused by prolonged thiamine (vitamin B1) deficiency. The prevalence of WE in different neuropathological studies varies between 0.4-2.8%, up to 12.5% in chronic alcoholic patients (1). Alcoholics are particularly predisposed to thiamine deficiency due to the low thiamine absorption rate at the mucosal level, the impaired hepatic function, and the alcohol-related raised thiamine metabolism (1-3). In addition, different clinical conditions can impair the correct absorption of thiamine: gastrointestinal surgery, prolonged vomiting, chemotherapy, systemic infectious and non-infectious diseases, and dietary imbalance (4).

According to the new Caine's classification criteria, clinical diagnosis of WE in alcoholics requires two of the following four signs: nutritional deficiency, ocular findings, ataxia, and mental status changes (5). To make a WE diagnosis promptly, Sechi and Serra have classified clinical symptoms and signs into three groups: symptoms common at presentation, uncommon at presentation, and late-stage symptoms (3). In this case report, we wanted to point out rare clinical signs in the late stage of the disease as well as the occurrence of stroke in WE and raise the awareness of this condition among adults.

CASE REPORT

A 39-year-old woman was admitted to Department of Emergency Neurology in an altered state of consciousness (Glasgow Coma Score 4), with myotic and non-reactive pupils, she had hypertonia with hyperexcitability of the myotatic stretch reflexes. On examination, she had stable vitals, albeit with pyrexia (38 °C), she was moderately underweight (BMI 17.9), tachycardic, and hypotensive. During the two weeks prior the admission, she had been hospitalized on Internal Medicine Department of a regional hospital due to hepatic dysfunction, deficit of albumin, folic acid, vitamins D and B12, electrolyte imbalance, and series of epileptic seizures. During that period, substitution therapy was started along with continuous infusions of glucose, and after that the working diagnosis of WE was established. From her past medical records, we found that she had the history of alcohol abuse for the past eight years, her food intake in the previous year was significantly decreased, and she demonstrated apathy with mild memory impairment for the past two years.

Upon admission to a tertiary healthcare facility, laboratory tests revealed mild alkalosis with pH 7.52, lactates were 0.9 (0.5-1.6 mmol/L), bicarbonates were elevated at 27.8 (22-26 mmol/L), blood magnesium was decreased at 0.47 (0.7-0.95 mmol/L) as well as blood potassium level at 2.8 (3.5-5.5 mmol/L), while levels of blood glucose (5.7 mmol/L), blood urea nitrogen (2.5 mmol/L) and blood creatinine (45 μmol/L) were within the reference range. Serum inflammation markers were elevated, with C-reactive protein (CRP) 87.3 mg/L (normal value is < 5 mg/L), and procalcitonin (PCT) level was 0.7 (normal value is < 0.1 ng/mL).

From the first day of hospitalization in our department, we registered transitory abnormal backward arching of the neck and body due to the severe muscle spasm (opistotonus) which lasted up to a few minutes, several times per day, with continuously present muscle hypertonia. The chest X-ray taken on admission demonstrated inflammation on the left side, which could explain the elevated CRP levels. Liver ultrasound revealed the signs of steatosis with coarse and fine-grained echostructure, while subsequent CT scan of the abdomen showed unevenly reduced density, particularly in the right lobe, with perfusion impairment, without clear focal changes. A moderate increase in liver enzymes (aspartate aminotransferase-73 (5-34 U/L), gamma-glutamyl transferase-228 (11-59 U/L)) was detected along with macrocytosis within chronic liver disease. Serum vitamin D < 11 (50-150 nmol/L) and folate 6.45 (8.6-45.3 nmol/L) levels were low, ammonium level was 13 (9.9-30 μmol/L), while the coagulation panel was unremarkable. The results of virologic workup, including tests for hepatitis A, B, C, D and E virus, human immunodeficiency virus (HIV) and cytomegalovirus (CMV), were unremarkable, too. The results of lumbar puncture and thyroid hormone levels were within normal limits. In all immunological analyses, the levels of antinuclear antibodies (ANA), antimitochondrial antibodies (AMA), anti-smooth muscle antibodies (SMA) and anti-parietal cell antibodies (APCA), antinuclear antibody HEp-2, anti-transglutaminase IgA antibody (TGA-IgA), rheumatoid factor (RF) were measured; complements C3

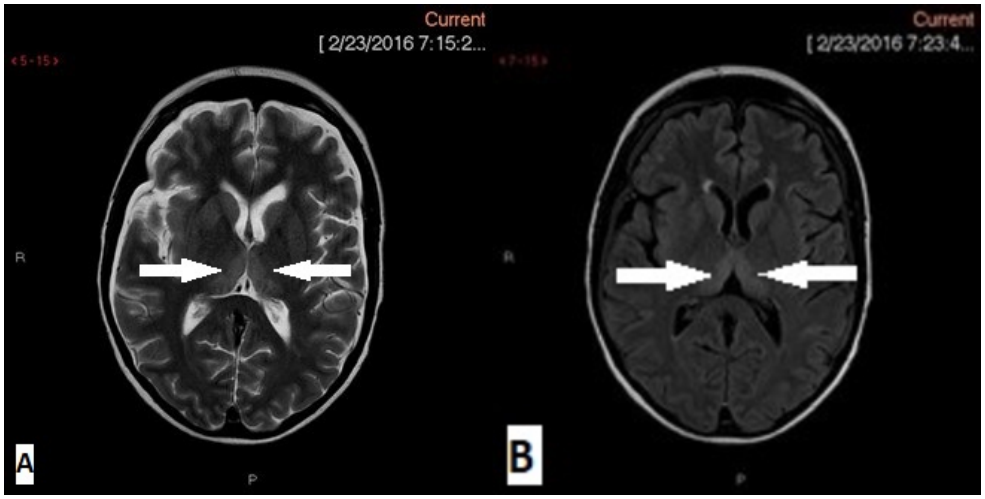

Figure 1. *(A, B): Axial T2-weighted/FLAIR images show symmetrically increased signal intensity in the dorsomedial thalami*

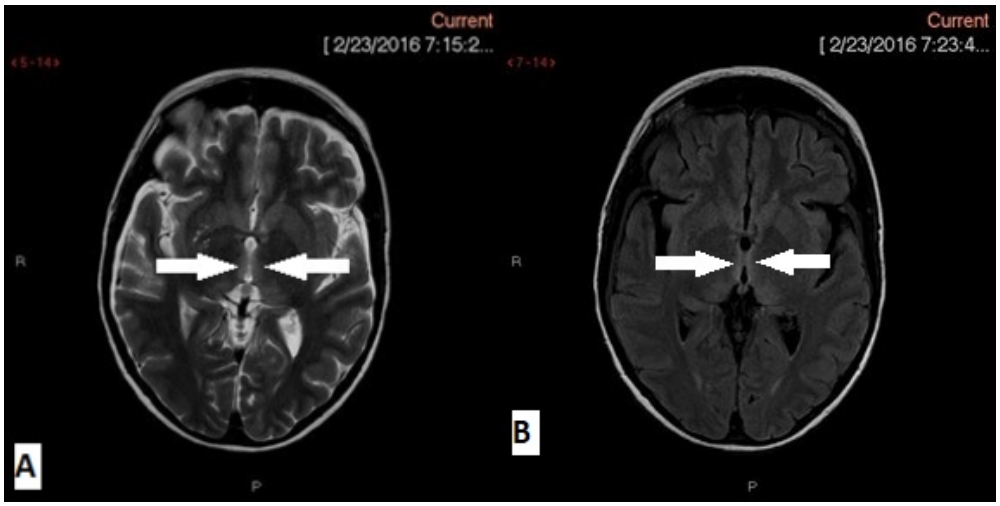

Figure 2. (A, B): *Axial T2-weighted/FLAIR images show symmetrically increased signal intensity in the periventricular region of the third ventricle*

and C4 were negative. Serum copper and ceruloplasmin levels were normal. A subsequent non-contrast CT brain scan was normal. On the fifth day of hospitalization, a brain MRI scan showed a symmetrical, hyperintense T2 signal in the region of dorsomedial thalami (Figure 1. (A,B) and around the third ventricle (Figure 2. A,B), as well as acute ischemic lesion of the pons (Figure 3. A,B). Electroencephalography (EEG) recording showed encephalopathic findings (Figure 4.).

During the initial period of hospitalization, the serum level of thiamine could not be determined due to technical issues. On the third day, we empirically started its intravenous administration in the three daily doses of 300 mg, combined with hepatoprotective and multivitamin therapy. After 24 hours, she improved dramatically, from a state of coma to obeying commands and opening her eyes appropriately. On the seventh day since the thiamine therapy began, we determined the serum normal range levels of 85 μg/L (28-85 μg/L).

In the following days, we registered only partial regression of neurological deficit, and the patient was conscious to somnolent, without verbal response and convulsions, having persisting muscle hypertonia with transient nuchal and lower spine rigidity, together with meanwhile registered horizontal nystagmus. After 12 days, the patient was transferred for further treatment to the secondary

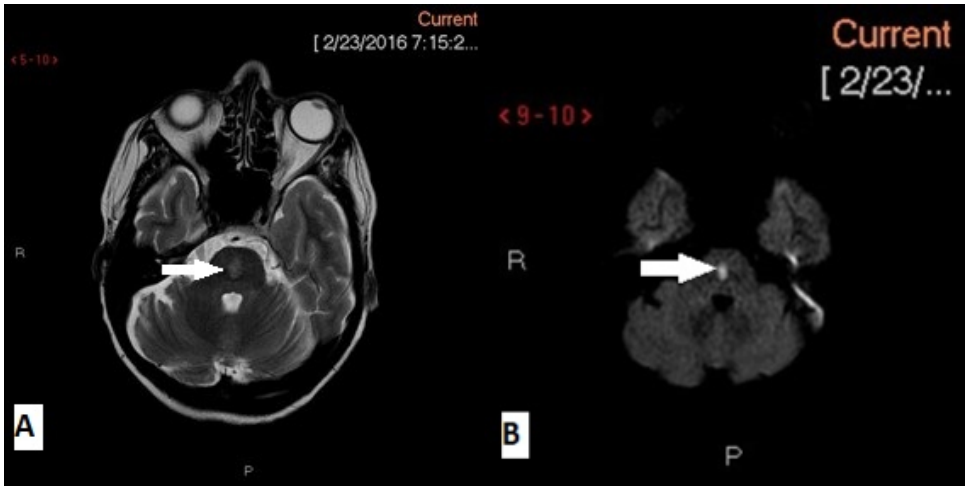

Figure 3. *(A, B): Axial T2-weighted/DWI images show minor high T2 and diffusion restriction abnormality in the central pons*

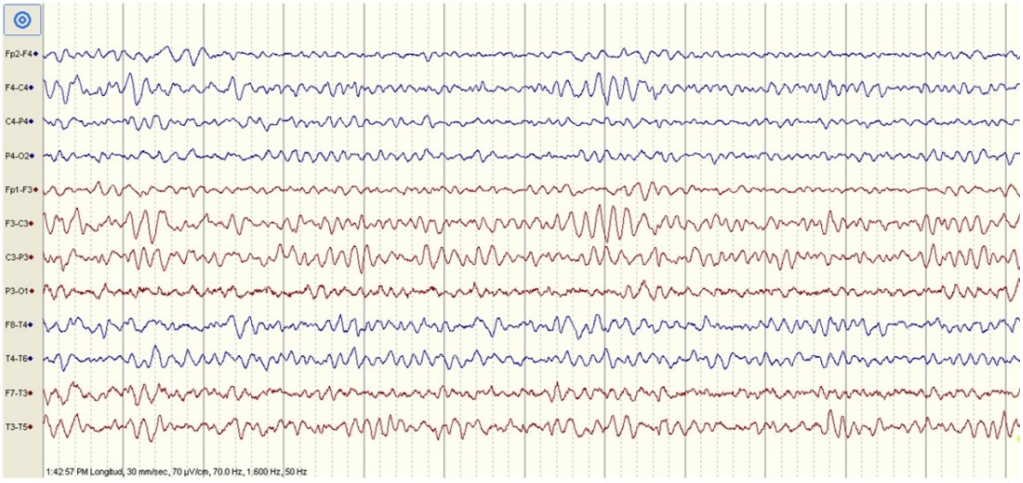

Figure 4. *The EEG shows moderate electrocortical dysrhythmia above bilateral centroparietal region, most prominent over the left side (calibration: 1 second/30 mm, 10 μV/mm)*

hospital care, where she died two weeks later. An autopsy was not performed.

Despite the lack of knowledge about temporal progression of neurological signs of WE, common symptoms (ocular abnormalities, mental status changes, incoordination of gait, and trunk ataxia) and uncommon symptoms or signs at presentation (hypotension and tachycardia, hypothermia, bilateral visual disturbances and papilledema, hearing loss, hallucinations, and behavioral disturbances) were established. Quantitative deterioration of consciousness (coma) is a hallmark of the late stage of disease (2-3 weeks after the onset of first symptoms) (3). A comatose state was described in the situations of untimely recognition of this disease and parenteral nutrition or glucose infusions without thiamine supplementation (which occurred in our patient) (6).

It has been considered that prolonged glucose administration without the addition of thiamine can be a risk factor for the clinical worsening in WE. The proposed underlining mechanism is that glucose activates glycolysis in which thiamine is consumed, which decreases its body supplies. At the cellular level, increase of lactates in the lesioned brain areas is due to anaerobic oxidation and lack of thiamine, along with underlying parenteral glucose administration. That is why the administration of glucose

before thiamine is not recommended (6, 7). On the other hand, Schabelman and Kuo, in reviewing the case reports, did not determine a clear influence of glucose infusions on the occurrence and deterioration of WE. A different methodological approach in a few case reports these authors analyzed was a limiting factor for the definite conclusion (8). In this case, our patient received protracted glucose infusions while being normoglycemic and without thiamine supplementation, while she was hospitalized in a regional medical center, which taken together could support previously mentioned hypothesis.

In the late phase of WE, patients can have seizures, hyperthermia caused by involvement of anterior hypothalamic regions, spastic paresis secondary to involvement of motor cortex or pyramidal tracts, hypertonia with nuchal and lower-spine rigidity, and choreic dyskinesias caused by damage to structures at mesopontine tegmentum (3). In our case, partial explanation of hypertonia could be found in the work of Zhang and colleagues who stated that the loss of neurons of anteroventral/ventrolateral (AVVL) and ventral posterolateral (VPL) thalamic nuclei, in the first hour from the beginning of epileptic seizures, could be responsible for hypertonia and opistotonus. In the next few hours, massive neuronal cell loss occurs in areas of other thalamic nuclei and mammillary bodies as well as in periaqueductal gray matter and in tegmentum of pons (9). According to other authors, a neuropathological mechanism of postural muscle tone loss would include metabolically caused structural lesions of mesopontine tegmental area and its reticular formation. Disfunction of dorsal reticulospinal tract causes disinhibition of the spinal cord which leads to hyperexcitability in opistotonus (3, 10). In our patient, MRI brain scan did not show lesions in this location of the brainstem, which does not exclude the existence of a lesion which could possibly be seen on MR spectroscopy or in postmortem examination.

Given that thiamine plays a key role in maintaining normal cellular and metabolic function in the brain, its deficiency leads to disruption of important enzyme pathways, increasing the vulnerability of the CNS (11). Specific places for neuronal damage are the ones with the highest need for thiamine such as gray matter of mamillary bodies, anterior and medial thalamic nuclei, upper and lower colliculi, and periventricular gray matter (12). As per literature, frequent histological changes can be found in bilateral, dorsal medial areas of thalamus as well (13). In our patient, we registered bilateral thalami and periventricular lesions, which pointed to brain structural selectivity and was in accord with the literature. The possible consequence of clinical or subclinical thiamine deficiency in alcoholism may contribute to significant variability in the range of alcoholism-related brain abnormalities detected. Possible explanations for this variability include individual alcohol use pattern (quantity, frequency, duration) and nutrition or hypothesis that different brain regions have different susceptibility to alcohol toxic effects, especially in the periventricular region where the blood-brain barrier is naturally thinner (4, 14, 15).

Another rarity relates to the ischemic pontine lesion detected in our patient. In the acute phase vascular congestion, microglial proliferation and petechial bleeding can be registered in WE, was a consequence of arteriolar and capillary dilatation (16), while the association of ischemia in WE has not been recorded in the literature frequently. Although ischemic stroke is not frequently described as a direct complication of Wernicke encephalopathy (17), certain mechanisms may explain this possible correlation. Namely, thiamine deficiency in WE leads to metabolic disturbances that can impair neuronal energy metabolism, particularly in structures such as the mammillary bodies, thalamus, and brainstem, which may increase the susceptibility of brain tissue to ischemic events. Moreover, patients with chronic alcoholism often have risk factors for ischemic stroke, including hypertension, cardiac arrhythmias, and coagulation disorders which can increase the likelihood of brain ischemic events.

Therefore, it is important to identify and appropriately manage all associated risk factors for cerebrovascular diseases in patients with Wernicke encephalopathy to reduce the likelihood of ischemic stroke.

CONCLUSION

Wernicke encephalopathy represents a clinical diagnosis which is based on physical and neurological examination along with neuroimaging. Apart from the already defined diagnostic criteria, in some cases, diagnosis of WE can be hard to establish due to rare and unrecognized clinical manifestations of this disease, such as muscle hypertonia and opistotonus. Using more detailed neuroimaging techniques such as functional MRI, contrast MRI, posi-

tron emission tomography and MR spectroscopy can allow physicians to examine both structural and functional changes that occur in patients with WE. With early recognition of frequent and unusual symptoms, especially in the late phase of disease, we can decrease morbidity, irreversible neurological damage or even death outcome, following the most important therapeutical guideline: thiamine should be given before glucose infusion to all patients with undetermined cause of comatose state.

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Conflict of interest

The authors have no relevant financial or non-financial interest to disclose.

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Simptomi kasne faze Vernikeove encefalopatije

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S A Ž E T A K

Uvod. Vernikeova encefalopatija je redak akutni/subakutni neurološki poremećaj koji je najčešće uzrokovan prolongiranom deficijencijom tiamina kod osoba koje hronično konzumiraju alkohol. Prema Kejnovim klasifikacionim kriterijumima, klinička dijagnoza ove encefalopatije podrazumeva najmanje dva od sledeća četiri znaka: nutritivna deficijencija, okulomotorna disfunkcija, ataksija i promene mentalnog statusa. Pojava različitih kliničkih simptoma i znakova može biti uslovljena i razvojem bolesti. Ovaj prikaz ukazuje na retke kliničke znakove u kasnoj fazi bolesti, kao i na posledice moguće lokalne hipoperfuzije moždanog stabla u vidu ishemijskog vaskularnog događaja.
Prikaz slučaja. Tridesetdevetogodišnja bolesnica (prethodno lečena u regionalnoj opštoj bolnici) primljena je na Odeljenje urgentne neurologije Univerzitetskog kliničkog centra Vojvodine sa podacima o seriji epileptičnih napada, poremećaju svesti, pojavi okulomotornih znakova, opistotonusu i kognitivnoj disfunkciji. Bolesnica je godinama konzumirala alkohol i bila u stanju nutritivne deficijencije. Dijagnoza je potvrđena tipičnim neuroimidžing nalazom i specifičnim laboratorijskim pretragama. Hipertonija i posledični opistotonus su kliničke manifestacije Vernikeove encefalopatije, dok je ishemijski moždani udar bio neočekivana pojava. Empirijska primena visokih doza tiamina uz dodatnu potpornu intenzivnu terapiju dovela do zadovoljavajućeg ishoda.
Zaključak. Vernikeova encefalopatija predstavlja kliničku dijagnozu koja se temelji na fizičkom i neurološkom pregledu uz neuroimidžing. Rano prepoznavanje kako čestih, tako i neuobičajenih simptoma, posebno u kasnoj fazi bolesti, moglo bi uticati na smanjenje morbiditeta i smrtnog ishoda. Neophodno je slediti važnu terapijsku smernicu: tiamin treba ordinirati pre infuzije rastvora glukoze svim bolesnicima sa neutvrđenim uzrokom promenjenog stanja svesti.

Ključne reči: **tiamin, Vernikeova encefalopatija, alkoholizam, opistotonus, moždani udar**

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Case report

The Case of Leptospirosis in a Female Patient with Hodgkin's Lymphoma

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SUMMARY

Introduction. Although we could not find the registered cases of leptospirosis co-occurring with lymphogranulomatosis, it is important to note that both diseases can affect the immune system. Therefore, the reported case is unique and will be interesting and useful for the physicians of various specialties.
Case report. We report a case of icterohemorrhagic form of leptospirosis with a fatal outcome in a woman with postmortem diagnosis of Hodgkin's lymphoma. Based on the findings of the autopsy, histological and immunohistochemical studies, it was established that the deceased suffered from Hodgkin's lymphoma during her life, classical form, reticular subtype with depletion of lymphoid tissue and extranodal spread in the liver, ovaries, and epicardium.
Conclusion. The combination of leptospirosis and lymphogranulomatosis was characterized by the complication of the diagnostic process, which should be taken into account by physicians of all specialties.

Keywords: **comorbid pathology, diagnosis, complication of the diagnostic process, clinical manifestations, autopsy**

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INTRODUCTION

Leptospirosis is a group of non-transmissible natural foci infections caused by spirochetes of the genus Leptospira and are rated first among zoonoses in terms of prevalence in natural and anthropogenic foci (1).

Hodgkin's lymphoma (HL) or lymphogranulomatosis is a disease with a primary lesion of the lymphatic system originating from the B-cells, in which a unique cellular microenvironment and activation of numerous signaling pathways support the proliferation of tumor cells and create the immunosuppressive environment for their survival (2, 3).

Although we could not find the registered cases of leptospirosis co-occurring with lymphogranulomatosis, it is important to note that both diseases can affect the immune system. Leptospirosis sometimes progresses to a more severe form known as Weil's disease (4), which can cause liver and kidney failure. Hodgkin's lymphoma can also weaken the immune system, making it more susceptible to infections such as leptospirosis. Therefore, the reported case is unique and will be interesting and useful for the physicians of various specialties.

We report a case of icterohemorrhagic form of leptospirosis with a fatal outcome in a woman with postmortem diagnosis of Hodgkin's lymphoma.

CASE REPORT

A 31-year-old female patient V. received treatment at the Infectious Disease Hospital. It was known from her past medical history that the disease had manifested a week after her husband independently carried out chemical rodent control at home.

Throughout the treatment period, the hemograms revealed anemia (Hb:73–107 g/L; RBC: 2.9–3.4 × 1012/L; color indicator—0.79-0.9), leukocytosis (40–47 × 109 /L), lymphopenia (3–10%), ESR: 25–50 mm/h. The findings of blood biochemistry test showed total bilirubin: 99–102.7 μmol/L; direct bilirubin: 85.8–86.5 μmol/L; indirect bilirubin: 12.5–16.9 μmol/L; total protein: 47–49 g/L; urea: 20.1–21.5 mmol/L; creatinine: 118–121 μmol/L; ALT: 33U/L; AST: 74 U/L.

The patient underwent a multislice chest and abdominal CT that revealed CT-signs of bilateral polysegmental pneumonia (CO-RADS 5), affecting ≥ 25% of the parenchyma, mediastinal lymphadenopathy, bilateral hydrothorax, a small amount of fluid in the pericardial cavity, fatty liver disease, hepatomegaly, abdominal lymphadenopathy, ascites.

The patient was diagnosed with leptospirosis, icterohemorrhagic form, laboratory confirmed Leptospira Gripothyphosae 1:50, 1:800, 1:400, severe course, toxic hepatitis with severe hepatocellular insufficiency, secondary nephropathy, anasarca.

Antibacterial (doxacycline) and detoxification treatment was started, intensive therapy and artificial lung ventilation were carried out, but her condition progressively worsened and the patient died. An autopsy of the dead body was authorized (informed consent and permission to publish this case were signed by the legal representatives).

The results of the autopsy, histological and bacteriological examination of the sectional material, the diagnosis of the icterohemorrhagic form of leptospirosis was confirmed. However, during the autopsy, enlarged lymph nodes of the thoracic and abdominal cavities were found, ranging in size from 1 to 4 cm in the largest diameter. They were conjoined into aggregations of a soft-elastic consistency; on the section they were colored gray-pink with crimson softened areas. In addition, a whitish soft nodule up to 4 mm in diameter was found under the epicardium on the lateral wall of the heart. The liver was enlarged and of flaccid consistency, speckled on the surface and on the section due to the presence of yellow and purplish-gray spots. The histological examination of the lymph nodes of the bifurcation of the trachea, mediastinum, pancreoduodenal zone, intestinal mesentery and hilum revealed that the histological structure was smoothed, follicles were not identified, massive necrosis and hemorrhages were pronounced. The preserved areas were infiltrated by the lymphoid atypical cells with the presence of isolated mitoses, including pathological ones. Large-sized cells with multilobed nuclei were noted among the cellular infiltrate (Figure 1). Similar cellular infiltrates were also found in the liver, stroma of the ovaries, and in the node under the epicardium.

Given that similar changes are characteristic of a number of lymphoproliferative diseases, for the purpose of differential diagnosis (5–8), the immunohistochemical study was conducted using markers CD 30, CD 138, CD 20, CD 3, Bcl2, Bcl6, Ki 67, OLA (Figure 2).

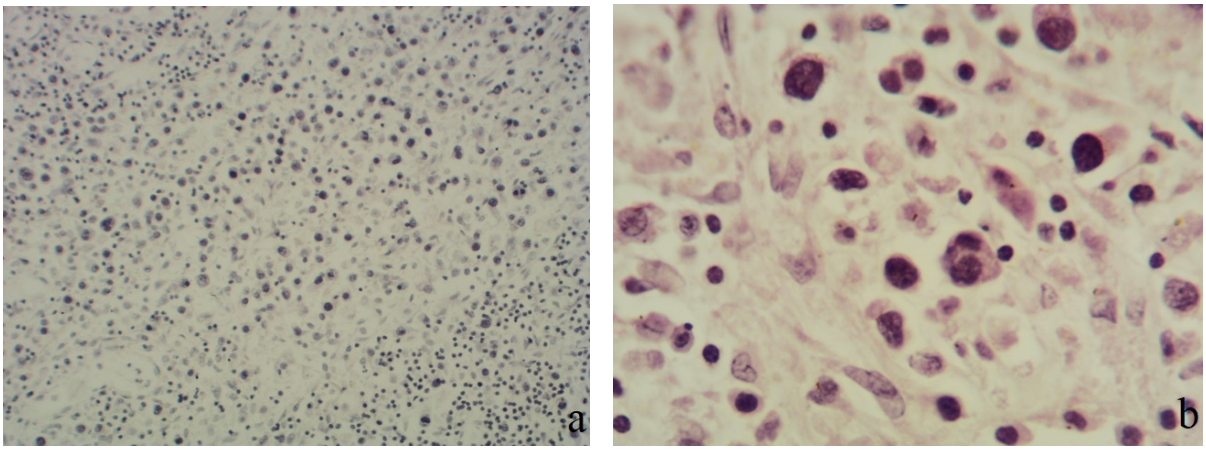
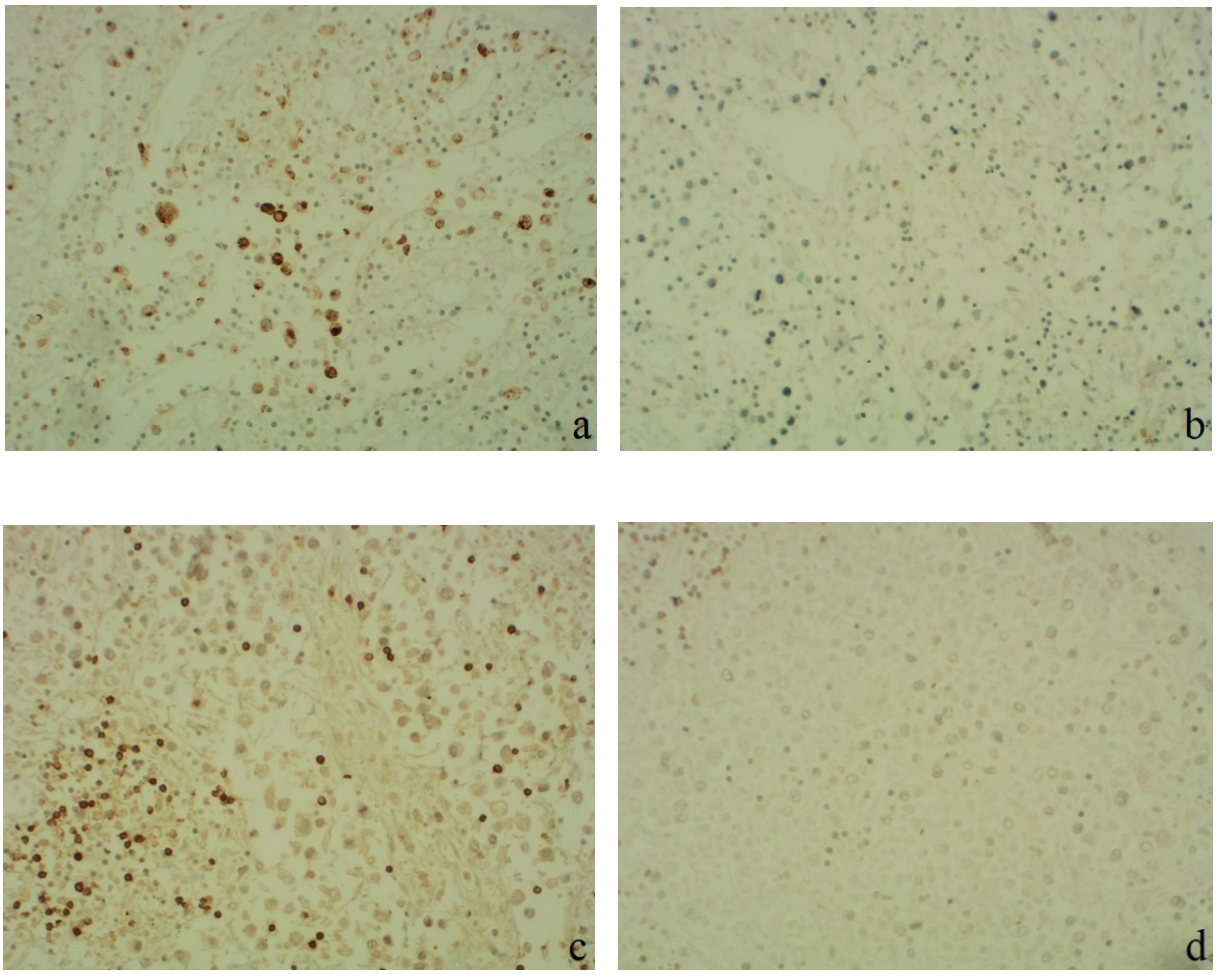


Figure. 1. *Microscopic changes in the lymph nodes of the deceased V.: a – general view, × 100; b – atypical polymorphic cells, × 400. Staining with hematoxylin and eosin*



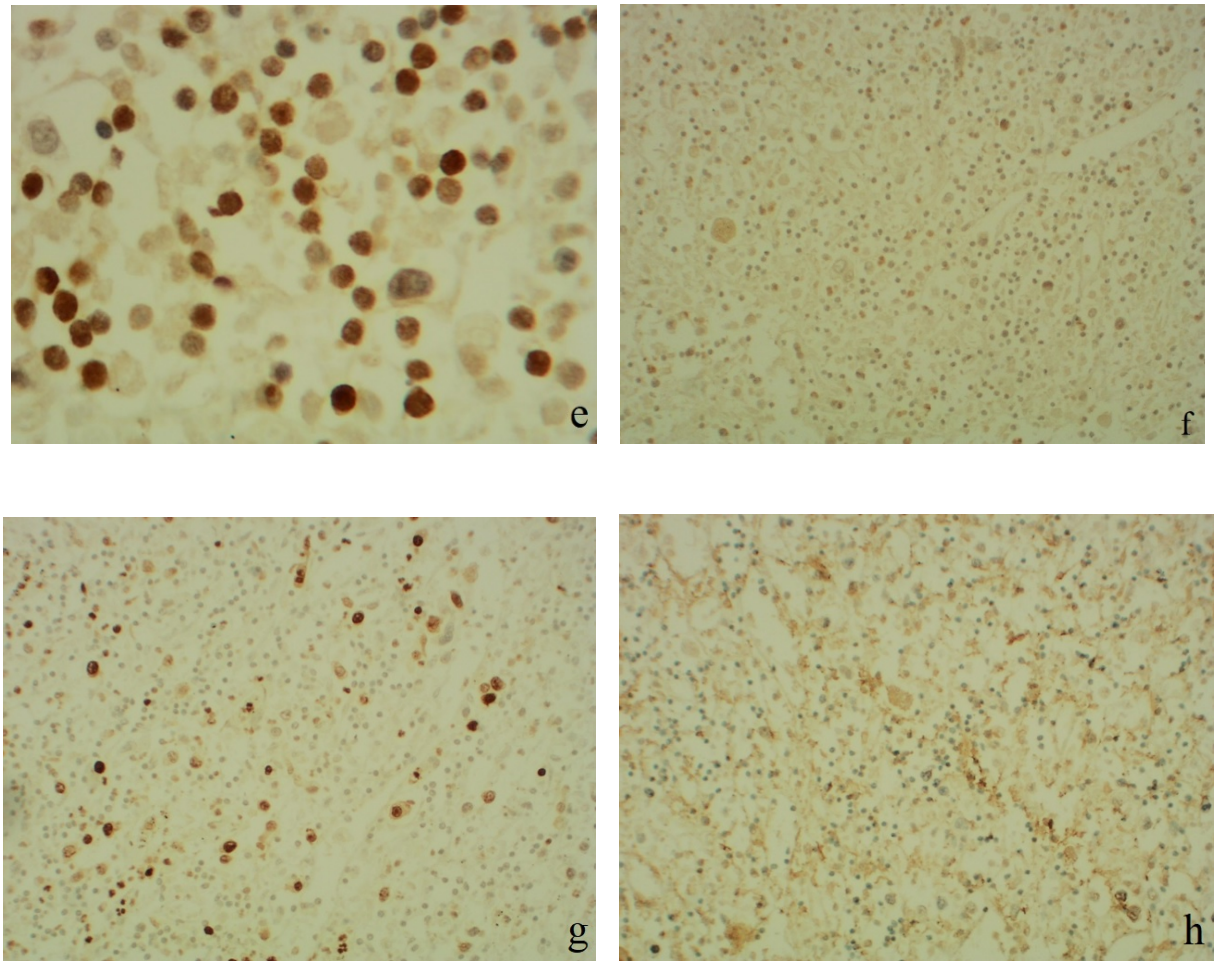


Figure. 2. *Immunohistochemical examination of the tissue of the lymph nodes of the deceased V.: a – CD 30 (× 100); b – CD 138 (× 100); c – CD 3 (× 100); d – CD 20 (× 100), e – Bcl2 (× 400), f – Bcl6 (× 100); g – Ki 67 (× 100); h – OLA (× 100)*

Based on the findings of the autopsy, histological and immunohistochemical studies, it was established that the deceased suffered from Hodgkin's lymphoma during her life, classical form, reticular subtype with depletion of lymphoid tissue and extranodal spread in the liver, ovaries, epicardium.

Thus, the patient had two diseases, which can be considered as concurred, because each of them could lead to the death of the patient.

DISCUSSION

A ubiquitous spread of leptospirosis is associated with a wide range of reservoir hosts of pathogenic Leptospira and animal species susceptible to them. Rats, mice, and dogs are carriers of Leptospira. The largest epidemic foci of leptospirosis are noted in countries with tropical and subtropical climates, where outbreaks involving hundreds and thousands of people are registered annually (1, 10). This infectious pathology has an important medical and social significance, since the annual morbidity constitute 1 million people, of which more than 50 thousand people die and many remain disabled (11).

Infection occurs through a direct contact with the reservoir host or indirectly through soil, urine of infected animals, and contaminated water. Icteric and anicteric forms of the disease are distinguished. Moreover, the mortality rate in the anicteric form is significantly lower compared to the icteric form. Ninety percent of cases of the disease are asymptomatic or have a mild form, and the symptoms are non-specific, which complicates the clinical diagnosis of leptospirosis (12, 13). Noteworthy, the course of leptospirosis can be affected by concomitant pathology. Therefore, it is important to study not only the clinical and morphological features and the improvement of the diagnostics of infectious di-

seases, but also the course, morphological and pathophysiological aspects of infectious diseases in coinfection and in the combination of an infectious disease with a non-infectious one, including tumor pathology (2, 3, 14, 15).

The diagnosis of leptospirosis was not difficult for the clinicians to make, based on the past medical history (the woman assisted her husband to remove rat corpses after deratization) and clinical and laboratory tests. However, clinicians sometimes cannot diagnose the second disease because of its atypical picture. Therefore, it is important to analyze the diagnostic steps that clinicians had to take to diagnose Hodgkin's lymphoma.

Due to the specific cellular interaction, two main forms of HL are to be distinguished: a classical and nodular one with a predominance of lymphocytes, which differ in prevalence, morphology, immunophenotype of cellular composition, and clinical picture. Classical Hodgkin's lymphoma, in turn, has four different subtypes: with nodular sclerosis, mixed-cell, with a predominance of lymphocytes, and with depletion of lymphoid tissue (4).

The deceased woman was diagnosed with Hodgkin's lymphoma with depletion of lymphoid tissue, which is the least common subtype with the incidence of less than 1% (17). The diagnosis of classical Hodgkin's lymphoma with lymphocyte depletion is difficult and is often detected at the advanced stages, and patients with this disease have the lowest survival rates, especially in the presence of adverse factors (18), which in this case were leptospirosis (extranodal disease), involvement of more than three groups of lymph nodes, lesions of mediastinal lymph nodes, high ESR.

Clinical manifestations of Hodgkin's lymphoma and leptospirosis commonly concur, which can lead to inaccurate diagnosis in cases of comorbid pathology. Both diseases can be manifested by an increase in body temperature, myalgia, headache, back and abdominal pain, anorexia, hepatomegaly, thrombocytopenia, and increased ESR. However, lymphadenopathy is a characteristic symptom of Hodgkin's lymphoma. Therefore, clinicians having detected enlarged mediastinal and abdominal lymph nodes, reveled by CT, should suspect another disease manifested by this symptom and perform a diagnostic puncture of lymph nodes in order to exclude lymphoproliferative disease (19, 20). After all, considering the pathogenesis of leptospirosis, lymphadenopathy is not characteristic of this disease.

Notwithstanding the absence of the direct link between leptospirosis and Hodgkin lymphoma, some studies have shown that infection with certain bacteria, viruses, or parasites can increase the risk for the development of lymphomas, including Hodgkin's lymphoma (8, 21). However, this correlation is not fully understood and more investigations are needed to determine its exact mechanisms.

CONCLUSION

For the first time ever, we described a case of comorbid pathology of leptospirosis and lymphogranulomatosis. The combination of these diseases was characterized by the complication of the diagnostic process, which should be taken into account by physicians of all specialties.

Conflict of interests

The authors have no existing or potential conflicts of interest to declare.

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Slučaj leptospiroze kod bolesnice sa Hodžkinovim limfomom

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S A Ž E T A K

Uvod. Iako nismo mogli pronaći registrovane slučajeve leptospiroze koja se javlja zajedno sa limfogranulomatozom, važno je napomenuti da obe bolesti mogu uticati na imunosistem; zbog toga je prijavljeni slučaj jedinstven i biće zanimljiv i koristan lekarima različitih specijalnosti.
Prikaz slučaja. Predstavljamo slučaj ikterohemoragijske forme leptospiroze sa fatalnim ishodom kod bolesnice sa dijagnozom Hodžkinovog limfoma postavljenom *post mortem*. Na osnovu nalaza obdukcije, histoloških i imunohistohemijskih studija utvrđeno je da je preminula bolesnica tokom života patila od klasičnog oblika Hodžkinovog limfoma retikularnog podtipa, praćenog smanjenjem limfoidnog tkiva i ekstranodalnim širenjem u jetru, jajnike i epikard.
Zaključak. Lekari svih specijalnosti treba da imaju na umu da kombinaciju leptospiroze i limfogranulomatoze karakteriše komplikacija dijagnostičkog procesa.

Klučne reči: **komorbidna patologija, dijagnoza, komplikacija dijagnostičkog procesa, kliničke manifestacije, autopsija**

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Case report

A Severe Case of Snakebite Envenoming by *Vipera ammodytes* in Winter

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SUMMARY

Introduction. Venomous snakebites in Serbia are neither too frequent nor extremely dangerous. Nevertheless, some can lead to complications or even death.
Case report. On December 24, 2023, a young man was admitted to hospital after being bitten by a snake, believed to be *Vipera ammodytes*. He was in a state of shock, unconscious, with unmeasurable blood pressure. Having received the proper treatment, the patient fully recovered.
Conclusion. The case described herein was the most severe clinical manifestation resulting from snakebite treated in the Užice General Hospital thus far and one of the most severe among the rare published cases of snakebites in Serbia. In our country, barely any information is available regarding venomous snake bites, so every peculiar case should be made public. We consider publishing this case even more important because it occurred at the beginning of winter when snakes should be inactive. To ensure better prevention and more efficient treatment of snakebites, appropriate transdisciplinary education has to be provided both to laypeople and medical workers.

Keywords: ***Vipera ammodytes*, shock, snake, treatment**

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INTRODUCTION

Snakebite is a special type of injury that occurs almost globally and is an especially severe issue in rural, low-income regions of (sub)tropical countries (1, 2). The injected venom causes a wide range of toxic effects, from local tissue damage to multi-organ failure or, finally, permanent disabilities or death (2–4).

In Serbia, as in other parts of the Balkan Peninsula and Europe, snakebites are neither too common nor extremely dangerous (5–7). Nevertheless, they do occur—only in the Užice General Hospital, 249 snakebite patients were admitted between 2006 and 2018 (8), and 64 people were treated for snakebites between 2019 and 2023 (unpublished). Unfortunately, data on snakebites are still not properly collected and systematized, not only in Serbia but in most of Europe (6, 9).

There are only three venomous snake species in Serbia, all protected by national and international legislation (10). The nose-horned viper, *Vipera ammodytes* (*V. ammodytes*), is widely distributed in our country, covering altitudes between 160 and 1,620 meters above the sea level (11). Reaching up to a meter, it is the largest viper in Europe and potentially the most dangerous (9, 12).

CASE REPORT

A 19-year-old male was admitted to the Užice General Hospital on December 24 at 4:18 p.m. due to a snake bite on his left hand. The bite happened approximately 40 minutes before admission, while he was hiking on the Cerovo hill near Arilje, a town in Western Serbia. Since the patient was unconscious, we obtained the hetero-anamnestic data from the accompanying person. The patient had no previous medical history and no known food or drug allergies. He was regularly vaccinated, including the tetanus vaccine five years before.

While climbing a steep slope, when he put his hands on the ground, our patient suddenly felt pain in the back of his left hand and saw a snake which he identified as *V. ammodytes*. An accompanying person immediately started the initial management of the bite injury by squeezing venom and blood out of the bite site. The patient experienced sweating and nausea, combined with abdominal pain in the first 15 minutes after the bite. He felt a tingling sensation and could not stretch his fingers. The patient was transported by car (about 30 minutes) to the nearest emergency room, where he received intramuscularly a single dose (5 ml) of the equine viper venom antiserum Viekvin® (13). Within 10 minutes, he was transported to the hospital. The patient vomited three times during the transport.

On admission, the patient was pale and unconscious. The vital parameters were as follows: blood pressure unmeasurable, heart rate 140/min, SO_2 95%, body temperature 36.5 °C. There was no hemorrhage.

The auscultatory findings of the lungs were normal. Clinical examination of the abdomen was normal. There was a visible bite mark between the index and middle finger of the left hand and oedema on the entire back of that hand.

The patient was immediately given single doses of adrenaline (0.5 mg/kg bw i.m.) and methylprednisolone (0.5 mg/kg bw i.m.). After 15–20 seconds the patient regained consciousness but was still disoriented. Spontaneous diuresis occurred after rehydration (fluid resuscitation with 20 mL/kg 0.9% sodium chloride i.v.). After 30 minutes the patient became oriented, and complained of pain in the hand, a tingling sensation, and paralysis of the fingers. An electrocardiogram revealed sinus tachycardia.

Laboratory findings upon admission to the hospital are given in Table 1.

Six hours after admission, the patient still felt weak with increased pain in his hand. The oedema extended, accompanied by pallor, bruising, and tenderness. Repeated laboratory findings indicated increases in CK (206 units/L) and LDH (310 units/L).

The patient was treated with antibiotic (ampicillin 500 mg/6 h PO) and symptomatic therapy.

Hospitalization lasted three days. Clinical and control laboratory findings before discharge were in the normal ranges of values. The local changes at the place of bite were in regression.

Table 1. *Results of laboratory tests upon admission to hospital. Boldface values are above and italicized below reference ranges*

Laboratory test	Result	Reference range
Hemoglobin	145 g/L	138–172 g/L
White blood cells (WBC)	**17.5 × 10^9/L**	4.5–1.0 × 10^9/L
Absolute neutrophil count (ANC)	**10.200 neutrophils/μl**	1.500–7.700 neutrophils/μl
Platelets	*133.000/μl*	150.000–450.000 platelets/μl
Erythrocytes	4.9 × 10^{12}/L	4.3–5.9 × 10^{12}/L
Blood sodium	*129 mEq/L*	135–145 mEq/L
Serum potassium	*3.2 mmol/l*	3.5–5.5 mEq/L
Calcium	**2.9 mmol/L**	2.2–2.7 mmol/L
Phosphorus	**4.8 mg/dL**	4.2–4.5 mg/dL
Blood glucose	**6.4 mmol/L**	3.9–5.6 mmol/L
Blood urea nitrogen (BUN)	6.2 mmol/L	1.8–7.1 mmol/L
Creatinine	98 μmol/L	61.9–114.9 μmol/L
Alanine aminotransferase (ALT)	25 units/L	0–35 U/L
Aspartate aminotransferase (AST)	21 U/L	0–35 U/L
Creatine kinase (CK)	180 U/L	33–211 U/L
CK–MB (creatine kinase-myocardial band)	12 U/L	< 24
Lactate dehydrogenase (LDH)	**260 U/L**	105–233 U/L
Fibrinogen	230 mg/dL	200–400 mg/dL
Prothrombin time	**14 seconds**	11–13.5 seconds

DISCUSSION

Evolved as a hunting/feeding aid and defense tool, snake venoms are virtually infinitely variable mixtures of inorganic components, enzymes, large proteins, toxic polypeptides, etc. that lead to a wide range of inflammatory, pharmacological, and toxicological effects (9, 14–18). They can cause a variety of potentially fatal clinical toxic syndromes affecting the nervous system (neurotoxicity), musculoskeletal system (myotoxicity), cardiovascular (cardiotoxicity), and blood clotting systems (hemotoxicity) (2, 19). Snakes from the genus *Vipera* produce venoms with predominating hemotoxic and cytotoxic but also necrotoxic components (9, 20). In Europe, potentially the most dangerous snake is the nose-horned viper, *V. ammodytes*, with four subspecies (21), listed by the World Health Organization as both Category 1 and 2 medically important (9). The venom of *V. ammodytes*, contrary to other members of the genus, was shown to have strong procoagulant potential (22), in addition to neurotoxic components (23, 24). Its bite, causing "local and systemic hemorrhage, tissue damage and neurotoxicity", can be fatal; the lethal potential of the venoms of *V. ammodytes* subspecies differs (9, 21, 25), as does the composition of venom within subspecies (16).

Our patient was admitted in a state of shock, which is not common for the clinical manifestations of snake bites in the Balkans (7), however, it occurs occasionally (26). The patient had tachycardia, but there was no arrhythmia, and there were no changes in the ECG. Since IgE was not elevated, the patient's condition resembled an anaphylactoid reaction.

One among only several published instances of snakebites in Serbia (27) was very severe, but in that case, the patient had been consuming alcohol, and several hours passed between the bite and admission to hospital. In the southernmost portions of Serbia (28), there were approximately 1/3 severe cases and one fatality among 264 patients. As in other reported complicated and fatal cases in the region (29, 30), these authors suspected a direct bite to the blood vessel without pronounced local signs but with a strong systemic reaction. There are also reports of severe complications resulting from *V. ammodytes* bite from Bulgaria and Greece (25, 31).

On admission, the blood pressure could not be measured in our patient. This was, however, quickly resolved with injections of adrenaline and methylprednisolone.

Our patient had thrombocytopenia, similar to cases described in the region (32). Major inducers of thrombocytopenia in *V. ammodytes* venom are snaclecs that cause platelet aggregation or their adhesion to blood vessel walls (23).

Venoms of Balkan vipers can cause transient renal failure and liver damage (26). Our patient had mild serum electrolyte imbalance which was quickly normalized.

The subjective sensation of tingling, paresthesia, and paralysis of the fingers in our patient can be explained by the neurotoxic effect of snake venom. Due to the presence of ammodytoxins, the venom of *V. ammodytes* affects even cranial nerves and directly the central nervous system, causing ptosis, ophthalmoplegia, dysphonia, dysphagia, swelling problems, neuromuscular weakness, unconsciousness, etc. (23).

Local manifestations of snakebite include excruciating pain and the appearance of immovable, tensely swollen, cold, and seemingly pulseless extremities. In our patient, local changes gradually regressed spontaneously.

Snake venom composition (and consequently its effect) varies geographically, individually, ontogenetically, depending on the dominant available prey, etc. (9, 33–35). There is a widespread belief that snake venom is more potent in spring than in other periods of the year (28), however, such assumptions of seasonal variations in venom yield and potency have rarely been confirmed (36, 37). Other investigations revealed only individual differences among snakes in the amount of produced venom (38).

Reactions to snake venoms depend on the snake (species, age, size), location of the bite, age, body mass index, health status of the bitten person, etc. (39). In our case, the patient was young and healthy hence such a strong reaction to venom was not expected.

Vipers are not uncommon in the Balkans. In Serbia, *V. ammodytes* has the widest distribution and has been recorded in the vicinity of Arilje (11). As ectotherms, reptiles are adapted to local climate and they respond to weather conditions. In temperate regions, snakes experience "winter dormancy", i.e. brumation. In *V. ammodytes*, this inactivity phase can last from September to April (40). Therefore, encounters with vipers at the beginning of winter are not expected. Nevertheless, there are reports to the media (41) or to/from our colleague herpetologists about snakes active during winter months (e-mail communication with R. Ajtić, PhD (rastko.ajtic@pmf.kg.ac.rs) on December 28, 2023, and A. Simović (alexandar.simovic@gmail.com) on December 29, 2023). Even if awakened from a hole covered with snow, a viper can quickly become agitated (42). In the neighboring countries somewhat warmer than Serbia—Croatia and Bosnia and Herzegovina—snake bites were recorded in January, November, and December (26, 43–45).

The correlation between weather/climate and snakebite incidence was shown previously (44, 46) and more thoroughly addressed recently (47–49). Climate changes influence both the snakes' distribution and annual activity rhythm, one of the changes being late brumation (50). Bearing in mind that the entire 2023 was globally "the warmest on record", and the second warmest in Europe, that we had "the warmest boreal autumn" (51), it is not surprising that reptiles delayed entering brumation.

Regarding snake bites, incomparably more published information exists for the neighboring countries than for Serbia (7). Also, for decades it has been suggested that reporting snakebites should be mandatory and all information should be collected in one place (9, 28, 52, 53). In Central and South-Eastern Europe (including Serbia), the initial steps were made (6), but with limitations. The most obvious is the low quantity of the obtained information. For the entire Serbia, the National Poison Centre collected data on 56 bites in three years. In the Užice region alone, 64 bites were recorded in four years.

Certain groups of nature-loving people are comparatively well-informed regarding venomous snakes (54), but we fear that most modern tourists do not possess enough quality information. To minimize the risk of snakebites and improve their treatment, it is necessary to initiate targeted transdisciplinary investigations into snake distribution, ecology, and behavior and to combine and publicize such information, to educate both residents and visitors in regions inhabited by venomous snakes (17, 49, 55, 56). Also, a guide for the clinical management of snakebites in Serbia could be produced, similar to that recently published for Italy (21). In addition, the media should be taught not to spread panic but to objectively report cases of snakebites.

CONCLUSION

Venomous snakebites occur in Serbia every year, but they are still not appropriately covered in the national scientific literature. The majority of the cases are mild or of medium severity, but sometimes, life-threatening symptoms develop. To better understand and treat such cases, it is necessary to publish reports of envenomations whenever possible.

The patient presented herein was young and healthy, but his reaction to venom was violent, which is not usual. Thanks to his companion and to timely and appropriate treatment by medical doctors, he fully recovered. This case highlights the fact that every snake bite should be treated individually, which demands experience.

Our patient was hiking as a tourist. Considering the increase in the presence of people in nature, often inexperienced, the education of hikers and tourist/mountain guides should be seriously considered. Communication between biologists and medical workers is essential. In "critical" regions, medical doctors should be educated to promptly recognize and deal with snakebites, and antivenom must be available. Also, the media should be educated not to spread panic but to objectively report cases of snakebites. With proper organization and wide cooperation, much can be done in snakebite prevention and treatment.

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Težak slučaj trovanja usled ujeda poskoka (*Vipera ammodytes*) u toku zime

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SAŽETAK

Uvod. Ujedi otrovnih zmija u Srbiji nisu ni preterano česti ni naročito opasni. Ipak, pojedini mogu dovesti do komplikacija ili čak smrti.
Prikaz slučaja. Dvadeset četvrtog decembra 2023. godine u bolnicu je primljen mladić koga je ujela zmija, najverovatnije poskok (*Vipera ammodytes*). Mladić je bio u stanju šoka, bez svesti, a njegov krvni pritisak nije mogao biti izmeren. Nakon adekvatnog zbrinjavanja i praćenja stanja, bolesnik se potpuno oporavio.
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Ključne reči: **poskok, šok, zmija, lečenje**

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