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CASE REPORTS / KAZUISTIKA

The human mind and conscience are amazing features that distinguish us, humans, from the animal world. Conscience, the personal ethics that guides our behavior, is at the foundation of the being of each of us. We follow our conscience and use its power to spread friendship and tolerance among people. Let us use wisdom to solve problems and inspire each other to behave in a similar way. April 5, the International Day of Conscience, will be marked this year under the slogan “Building a Culture of Peace”.

Ljudski um i savest su zadivljujuće osobine koje razlikuju nas, ljude, od sveta životinja. Savest, lična etika koja nam je vodič za ponašanje, u temeljima je bića svakog od nas. Sledimo svoju savest i koristimo njenu moć za širenje prijateljstva i tolerancije medju ljudima. Upotrebimo mudrost za rešavanje problema i inspirišimo jedni druge za slično ponašanje. Međunarodni dan savesti, 05. april, ove godine će biti obeležen pod sloganom „Gradimo kulturu mira“.

GENERAL REVIEW

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Oral microbiome, COVID-19 and probiotics

Oralni mikrobiom, COVID-19 i probiotici

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Key words:
gastrointestinal microbiome; microbiota; mouth; COVID-19; probiotics; virus diseases; immunity; symbiosis.

Ključne reči:
mikrobiom, gastrointestinalni; mikrobiota; usta; COVID-19; probiotici; virusne bolesti; imunitet; simbioza.

Introduction

The COVID-19 pandemic is an ongoing global pandemic that seriously endangers human life and health. Clinical presentation of this disease varies from completely asymptomatic or mild infection to severe complications, post-COVID syndrome, and even lethal outcome [1].

The etiologic agent of COVID-19 disease is the SARS-CoV-2 virus, an RNA-positive single-stranded virus from the *Coronaviridae* family. Even though coronaviruses primarily cause zoonotic infections, there are currently seven strains that can cause an infection in humans [2]. There are five different variants resulting from genetic evolution that have been identified since the onset of the pandemic: Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) [3].

The SARS-CoV-2 virus is one of many respiratory viruses where the oropharynx is the primary site of entry and replication [4]. It binds to angiotensin-converting enzyme 2 (ACE-2) receptors *via* its glycoprotein extension (S protein) [4–6]. These receptors are present on the tongue, oral and nasal mucosa, salivary glands, and nasopharynx [7–11]. The study of Xu et al. [12] showed higher ACE-2 expression in small salivary glands than in lungs during COVID-19 disease, proving that salivary glands represent a significant virus reservoir [13].

Since the beginning of the COVID-19 pandemic, basic preventive measures have been applied to prevent the transmission of the virus (hand disinfection, wearing face masks, social distancing, and quarantine). Thirteen different official treatment protocols were introduced, though currently, there is no optimal treatment [1]. Antiviral therapy, vaccines, and immunomodulating agents are widely used in order to reduce disease severity, especially in patients with an increased risk of developing a severe clinical form of the disease [14]. While searching for the most effective treatment against the COVID-19 infection, scientists and clinicians have also applied alternative possibilities for improving immunity [15]. Recent research data have revealed the interaction of intestinal and respiratory systems in immunity induction, acting as local and systematic modulators of inflammation [16].

The human microbiome is important for blocking inflammation and immunity regulation. The impact of oral microbiome (OM) dysbiosis in patients with COVID-19, which can directly or indirectly favor the development of the infection and affect the pathogenesis of the disease, has been recognized. The human microbiome plays a significant role in the immune response of the host to respiratory viral infections. The modulation of local and systemic immune reactions by using probiotics is one of the most promising effects of probiotics on overall human health [15]. The presence and registration of dominant microbial communities in an individual's OM can enable personalized therapy that aims at restoring the microbiome and preventing the occurrence of many diseases in the future [17].

Many studies on COVID-19 published in the past three years have examined the exact mechanism of virus replication at the primary site of infection, as well as the role of OM on SARS-CoV-2 virus binding capacity and infection development [4, 5, 9, 18–25]. The goal of this review was to evaluate the role of OM in the prevention of SARS-Cov-2 virus infection and its impact on the severity of COVID-19 clinical presentation. In addition, the aim of this literature review was to present possible preventive and therapeutic applications of probiotics which were used as one of the

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remedies against SARS-Cov-2 virus infection. This review focuses on the analysis of oral microbiota during the COVID-19 infection and gives us new insights into the relationship between microbiota and probiotics.

Oral microbiome

The human OM is the genome of all microorganisms which inhabit the oral cavity. The term "microbiota" refers to a specific and unique composition of microbial population that affects health and varies from person to person. In many studies, these two terms are equated [26–28].

Oral flora is the second largest and one of the most diverse microbiomes in the human body, right after the intestines, which weigh about 2 kg [29–32]. The composition of oral flora is heterogeneous and contains over 1,000 different bacterial species, viruses, fungi, helminths, protozoa, and archaea that persist in mutual balance but also in symbiosis with the host [23, 33–35]. Each person has a complex of microorganisms, and everyone carries an individual microbiome that develops over a lifetime. The composition and diversity of the OM can be influenced by the following: the duration of pregnancy, delivery method, breastfeeding, genetic factors (sibling microbiota profiles are more similar when compared to the profiles of persons who are not related), environmental conditions (oral hygiene, saliva quality, and quantity), habits (tobacco, alcohol, stress, etc.), diet, certain drugs (antibiotics, antacids, etc.), age (three stages of evolutionary development: childhood, adulthood, and old age), and individual general health [4, 36–42].

The most common species of bacteria in the OM are representatives of the genera *Bacteroides*, *Synergistes*, *Gemella*, *Granulicatella*, *Streptococcus*, *Veillonella*, the phyla Actinobacteria, Proteobacteria, Tenericutes, Firmicutes, and Spirochaetes, while oral viruses are mainly composed of eukaryotic viruses such as *Herpesviridae*, *Papillomaviridae*, and *Anelloviridae* [human papillomavirus (HPV), human cytomegalovirus (CMV), herpes simplex virus type-1 (HSV-1), and Epstein-Barr virus (EBV)] [32, 43]. *Myoviridae* and *Podoviridae* belong to lytic viruses (they rapidly degrade their bacterial hosts), while *Siphoviridae* are lysogenic viruses that are in balance with the host bacteria. Oral viruses represent a restricting factor for bacterial growth and can control bacterial oral populations [44, 45]. Research by Peters et al. [46] described 154 species of commensal fungi and confirmed that the *Candida* genus was most commonly present in 70% of healthy patients. Recent studies revealed the commensal presence of different genera of protozoa, helminths, and archaea. Nonetheless, their pathogenic potential in the development of oral diseases is still unexplored [47–49].

Importance of oral microbiome in oral and general health

The oropharyngeal microflora in a healthy host maintains balanced symbiotic relationships defined as "microbial homeostasis" (eubiosis) [32]. The OM is exposed to frequent daily fluctuations that can lead to microbial imbalance (dysbiosis) [50, 51]. Microbial balance in the oral cavity is necessary because it enables equilibrium between beneficial and harmful microorganisms that interact with each other and can have an inhibitory, stimulating, or synergistic effect on each other [32, 50, 51]. The OM contributes to the development of the local immune system. However, its imbalance, along with the complex interaction with the host resistance and various environmental factors, creates conditions for the development of various oral or systemic diseases (Figure 1) [17, 52, 53]. During dysbiosis, the following three changes occur: loss of

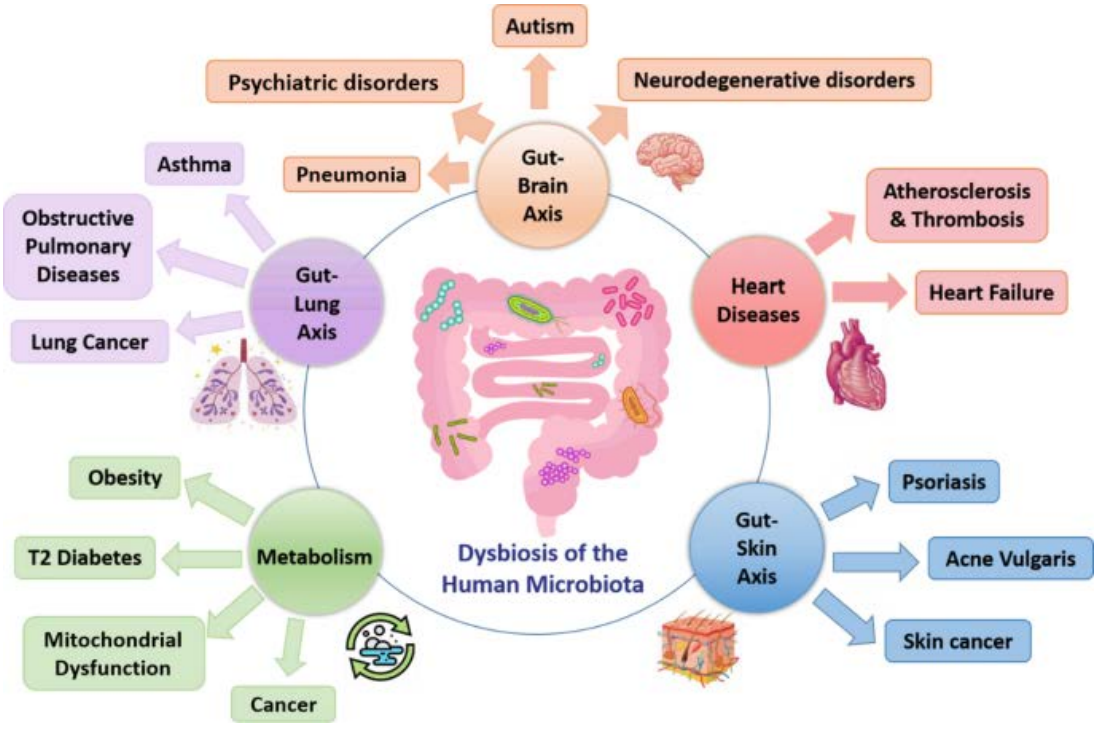

Fig. 1 - Impact of microbiota dysbiosis on the overall health of humans [52].

microbial diversity, loss of beneficial microorganisms, and increase in pathogenic microorganisms that are not mutually exclusive and can occur simultaneously [54]. As a result, potentiating cariogenic and inflammatory bacterial genomes [33, 55] make perfect conditions for the development of diseases such as caries [56–59], periodontal diseases [60–64], and malignant alterations of oral tissues [65–67].

Many oral microorganisms enter the digestive tract through saliva, thus changing the intestinal microbiome, which plays an important role in digestion and absorption of nutrients, formation of a protective barrier against pathogens, development and regulation of the immune system, enzyme and vitamin production, control of inflammatory reactions, and neuro and psychological regulation [35]. Consequently, the connection between the oropharyngeal and intestinal microbiome sheds new light on inflammatory processes and their therapy. The link between oral and general health is well documented in the literature [35, 50, 52, 68–76]. Chronic oral infections lead to the release of proinflammatory mediators, i.e., cytokines that further propagate inflammatory processes and increase the risk of muscle and digestive problems [61, 72], bronchopulmonary diseases [37, 73], rheumatoid arthritis (RA) [53, 67], complications during pregnancy and childbirth [38, 41, 64, 71, 75], cardiovascular diseases [61, 67, 69, 70, 75, 76], obesity [42], liver disease [67], oral cancer [65], pancreatic cancer [77], type 2 diabetes [67, 68], Parkinson's disease [74], psychiatric disorders [78], and colorectal cancer [67]. Each disease is characterized by unique oral and intestinal microbial changes [79]. Microbiome rebalancing is closely related to the recovery from the primary disease, which proves the significant role of the microbiome in healing [72, 80].

Maintaining good oral hygiene is, therefore, important in controlling oral bacterial status, maintaining or restoring symbiotic homeostasis, as well as preventing the spread of oral pathogens to other parts of the body [81–83].

Disruption of the oral microbiome during and after SARS-CoV-2 virus infection

Human OM can have a great influence on the regulation of innate and acquired immunity to viral infections [4], which is especially important for viruses that enter the body through the oropharynx [5, 23, 84–86]. Aerosol respiratory viruses encounter the OM of the upper respiratory tract and favor its dysbiosis and disease progression [87, 88] (e.g., the microbiome in patients with influenza is characterized by the abundance of genus *Pseudomonas*) [75]. Oral dysbiosis promotes respiratory infections directly by increasing pathogenic bacteria and aspirating oral pathogens into the respiratory organs. Indirectly, it alters the immune response of respiratory epithelium and promotes the adhesion of pathogens, cytokine secretion, and production of enzymes that interfere with pathogen clearance [23, 89]. Even in the case of SARS-CoV-2 virus infection, oral dysbiosis may favor the development of infection by these mechanisms [4]. On the other hand, OM can also contribute to the regulation of immunity and inflammation blockade [13, 30, 84, 90]. The study of Pfeiffer and Sonnenburg [91] showed that oral microbiota commensals could also produce antiviral compounds (defensins) against several viral genera (*Adenovirus*, *Herpesvirus*, *Papillomavirus*, *Orthomyxovirus*, and *Coronavirus*).

The oropharyngeal and intestinal microbiota have the possibility of co-infection with microorganisms originating from the oral cavity. The correlation between the imbalance of OM and the increased number of dysbiotic species may serve as predictive factors for COVID-19 disease [5, 35, 79, 92–104]. Ward et al. [24] showed that the severity of COVID-19 disease could be predicted according to the composition of the intestinal or OM. Two pathogens, *Porphyromonas endodontalis* in oral and *Enterococcus faecalis* in the intestinal microbiome, may serve as a predictor of the severity of SARS-CoV-2 infection. The authors suggested that the key to prioritizing patients who need urgent treatment is the identification of biomarkers that can predict clinical outcomes of COVID-19 disease [24].

Haran et al. [23] described how the dysbiosis of the inflammatory type of OM, characterized by the members of genera *Prevotella* and *Veillonella*, may play an important role in prolonging the duration of COVID-19 symptoms. The genus *Veillonella* are gram-negative anaerobic cocci that produce large amounts of lipopolysaccharides and may be an additional co-infectious agent (especially *Veillonella dispar* and *Veillonella infantium*) [98]. The genus *Prevotella* is highly inflammatory and affects the promotion of SARS-CoV-2 infection, thus worsening the severity of the COVID-19 clinical presentation [105–107]. Haran et al. [23] emphasized the importance of the presence of gram-negative bacteria with a liposaccharide component in the capsule (*Leptotrichia* and various species of genus *Veillonella*) which can have proinflammatory effects and cause systemic damage and neuroinflammation. Due to OM dysbiosis and the presence of pathogens that promote chronic inflammation (*Leptotrichia*, *Prevotella*, and *Fusobacterium*), myalgic encephalomyelitis and symptoms of neurological diseases (confusion, disorientation, slow thinking, and poor concentration after six months) may occur in COVID-19 [102–104]. The present commensals (*Prevotella* and *Neisseria*) in OM can act as a local probiotic and counteract the SARS-CoV-2 virus [17]. Likewise, the higher relative abundance of the *Rothia* genus in the patient's oral flora affects the occurrence of COVID-19 complications [108].

Ren et al. [79] identified specific microbial markers of oral microbiota in patients with COVID-19 but also in recovered patients. The results of this study showed compositional and functional changes in OM of COVID-19 patients. During infection, there is an overall decrease in the diversity of oral microorganisms. The number of bacteria that produce lipopolysaccharides was increased, while the number of bacteria that produce butyric acid was decreased. Thus, by secreting lipids into the bloodstream, microbiome dysbiosis may affect the progression of COVID-19. Ren et al. [79] also noticed a better prognosis in patients with severe COVID-19 who had an OM enriched with *Streptococcus* (*S. parasanguinis*). Oral dysbiosis persisted even after the recovery from COVID-19 infection when a constant increase in *Porphyromonas* and *Haemophilus* gen-

era and a decrease in *Leptotrichia*, *Megasphaera*, and *Selenomonas* (*Megasphaera* is a cariogenic bacterium) [106] genera was detected.

Furthermore, an imbalance in the relative numbers of different bacterial strains and the genera *Enterococcus* and *Enterobacter* were present only in patients with COVID-19 (not observed in the control group of healthy patients) [5]. Ward et al. [24] showed that higher quantities of *Porphyromonas endodontalis* are correlated with an increase in severe stages of COVID-19, while higher quantities of *Muribaculum intestinale* are linked with moderate cases. Cox et al. [95] also highlighted the impact of coinfections on the clinical presentation and mortality of patients with COVID-19. They indicated an association between cariogenic and oral pathogenic bacteria and complications of COVID-19. According to the earlier literature evidence, these bacteria are involved in the pathogenesis of respiratory and chronic inflammatory systemic diseases (type 2 diabetes mellitus, hypertension, cardiovascular disease), which are also the most common comorbidities associated with the risk of severe complications and death from COVID-19 [97, 109, 110]. Marouf et al. [101] also observed an abundance of periodontopathogenic bacteria in COVID-19 patients and demonstrated that the presence of periodontitis was associated with a 3.5-fold higher risk of admission to intensive care units, a 4.5-fold higher risk of assisted ventilation, and an 8.81-fold higher risk of mortality independent of other concomitant risk factors. On the other hand, numerous studies reported that interventions aimed at boosting oral hygiene in patients with pneumonia have significantly improved the clinical picture and reduced mortality [23, 35, 105, 107].

The exact genome of oral flora in patients with COVID-19 is still the focus of scientific interest. Iebba et al. [20] were among the first who had described the bacterial component of OM in patients with COVID-19, pointing to the importance of fungi and viruses in defining individual sensitivity. By examining an oropharyngeal swab, Ai et al. [93] found that more than half of COVID-19 patients had co-infection with another virus, such as influenza A or B, rhinoviruses, enteroviruses, or respiratory syncytial virus. Soffritti et al. [5] investigated an association between OM profile and severity of COVID-19 clinical presentation and observed a significant increase in *Herpesviridae* viruses, EBV, and HSV-1. EBV infection in patients with COVID-19 has been associated with an increased risk of severe COVID-19 symptoms as well as a fatal outcome [96, 99].

Changes have also been observed in the fungal part of the microbiome, with the appearance of *Aspergillus*, *Nakaseomices*, and *Malassezia spp*., *Candida albicans*, *Saccharomyces cerevisiae*, *Aspergillus fumigatus*, and *Malassezia restricta* (OM of healthy patients consists only of *Candida* and *Candida cerevisiae*, *Aspergillus fumigatus*, and *Malassezia restricta*) [5]. According to Jasinski-Bergner et al. [109], these changes in COVID-19 patients could be an inducing factor influencing the onset of SARS-CoV-2 virus infection because dysbiosis facilitates the activation or reactivation of oral pathogens, which can further impair proper immune control and lead to deterioration of immune response effectiveness.

Ward et al. [24] found that the composition of OM has a high accuracy of COVID-19 severity prediction (84% accuracy of predicting fatal outcome).

Application of probiotics

With the emergence of increasing antibiotic resistance, new concepts for the prevention and therapy of multidrug-resistant infections are proposed by causing the microbiological shift of the endogenous microbiota. For this reason, research into bactericidal action and antiviral factors of probiotics became the focus of modern interest [107, 111–113].

Positive effects of probiotics are primarily observed in the treatment of gastrointestinal infections but also in the prevention of various pathological conditions in the field of gastroenterology, allergology, internal medicine, oncology, oral medicine, pediatrics, infectiology, and psychiatry [104–107, 109–114].

Modern therapeutic procedures have introduced changes in treatment protocols with a tendency to establish a healthy environment to prevent the development of opportunistic infections and recover the OM. Probiotics are living microorganisms (so-called "good" bacteria) that, if applied in an adequate amount, could establish and maintain the ecological balance of microflora. They are safe, non-pathogenic, non-invasive, and non-carcinogenic strains that can perform recolonization and restore symbiosis between the host and the disturbed microbiota [115]. Several bacterial genera are most often used as probiotics: *Bifidobacterium*, *Lactobacillus*, *Bacillus*, and *Pediococcus*. The strains used most often *are Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Lactobacillus rhamnosus*. In addition, fungi can be used as probiotics [112–115].

Probiotics act in complex multifactorial ways. Probiotic bacteria can interfere with the absorption process by directly binding to the virus or inhibiting the entry into the epithelial cells by blocking the host receptor [25]. Probiotics can also compete with nutrient pathogens, produce antimicrobial agents, strengthen the intestinal epithelial barrier, and modulate the immune system of the host [116–118]. The performance of probiotics can be twofold – immunostimulatory and immunoregulatory. A group of immunostimulatory probiotics affects the proliferation of T helper (Th) 1 cells and stimulates the production of interleukin (IL)-12, which induces the production of interferon-gamma (IFN-γ) in natural killer cells. Immunoregulatory probiotics stimulate regulatory T cells but also suppress proinflammatory responses by inducing IL-10 [117]. Experimental animal models showed that balancing cellular and humoral immune responses mitigates the effects of a "cytokine storm" [25, 119].

The significant role of probiotics in maintaining homeostasis of the upper respiratory tract microbiome was proven in multiple studies. In addition, it was revealed that oropharyngeal probiotics are very effective in maintaining immune

system stability and protecting against viral infections [120–122]. Direct and indirect efficiency of various probiotic strains (e.g., *Lactococcus lactis* JCM 5805 and *Bacteroides breve* IIT4064) has been proven against influenza virus [123]. Probiotic bacteria release various substances, such as bacteriocins, biosurfactants, lactic acid, hydrogen peroxide, nitric oxide, and organic acids, which can inhibit virus proliferation [25]. *Lactobacillus* genus produces lactic acid as an antiviral inhibitory metabolite, thus preventing secondary infections [124]. Furthermore, like the genus *Bifidobacterium*, *Lactobacillus* genus can capture the virus and interfere with the binding of the virus to the receptors of the host cell [125, 126]. Nisin and peptide P18 are bacteriocins with antiviral effects against influenza A virus [127]. Apart from bacteriocin production, the antiviral ability of oropharyngeal probiotics is also maintained by the stimulatory effect on the innate immune response, which is manifested by an increase in the IFN-γ levels in human saliva ten hours after oral administration of *Streptococcus salivarius* (strain K12) lozenge [128]. Probiotic strains of genera *Lactobacillus* and *Bifidobacterium* (such as *Lactobacillus reuteri* ATCC 55730, *Lactobacillus paracasei*, *Lactobacillus casei* 431, *Lactobacillus fermentum* PCC, and *Bifidobacterium infantis* 35624) are significant factors in generating immunomodulatory responses during various infections [25, 129]. In addition, probiotic bacteria have antioxidant potential in neutralizing free radicals. The strains *Lactobacillus rhamnosus* GG, *Lactobacillus plantarum* CAI6, *Clostridium butyricum* MIIAIRI 588, and strains in VSL#3® increase total antioxidant capacity [130]. Moreover, by participating in the formation of redox homeostasis, probiotics can inhibit the progression of COVID-19 disease [25].

Probiotics and COVID-19

The proven effectiveness of probiotics both in the treatment and prevention of viral infections was the rationale behind their use in patients with SARS-Cov-2 virus infection [25, 28, 131–135].

In the last three years, studies dealing with the importance and benefits of probiotics in the prevention and treatment of COVID-19 have been conducted (Table 1) [21, 135-143]. The use of probiotics, along with other therapies, led to a shorter and easier clinical presentation, with reduced severity of gastrointestinal and respiratory symptoms, a lower percentage of smell and taste disorders, and less frequent symptoms of post-COVID syndrome [125–143]. However, in patients on corticosteroid therapy, probiotic supplemental therapy is contraindicated due to their primary disease [135].

The first study that proved the positive effects of probiotics in the treatment of COVID-19 infection was the 2020 Wuhan study [21]. The use of the probiotic strain ENT-K12 (*Streptococcus thermophilus*) among medical workers in institutions for COVID-19 treatment has reduced the possibility of respiratory infection and lethal outcomes by 80%. This probiotic strain locally releases two antibiotics (salivaricin A2 and B) and reduces the possibility of colonization of β-hemolytic group A streptococci, including *Streptococcus pyogenes* (a bacterial pathogen that causes coinfection during viral infection). The use of probiotics has also reduced the use of antibiotics among the respondents by more than 90% [21].

Block [144] suggested the concomitant use of probiotics in patients with COVID-19, treated with azithromycin, to reduce the risk of hypercolonization of *Candida albicans* strains. Nutritional support with probiotic strains of *Lactobacillus acidophilus*, *Bifidobacterium*, and *Saccharomyces boulardii*, along with minerals and vitamins, has reduced the complications of massive antibiotic therapy [145–147].

D'Ettorre et al. [135] compared the incidence of respiratory failure and control of symptoms, after probiotic therapy, with different *Streptococcus*, *Lactobacillus*, and *Bifidobacterium* strains. The use of probiotics was associated with a lower risk of respiratory failure and faster control of COVID-19 symptoms (especially diarrhea). In patients with a severe clinical picture, the immunomodulatory effects of probiotics may be relevant for the prevention of acute respiratory distress syndrome and multiple organ failure as a complication of cytokine storm [135].

Ceccarelli et al. [147] observed a lower mortality rate after the use of probiotic strains of genera *Streptococcus*, *Bifidobacterium*, and *Lactobacillus* during COVID-19, but with longer hospital stays. However, research by Bozkurt and Bilen [142] showed that in patients with moderate and severe COVID-19 symptoms, an additional therapeutic dose of the probiotic strain *Bifidobacterium animalis* resulted in a shorter hospital stay and lower mortality rates.

Probiotics mechanism of action in COVID-19

The mechanism of probiotic protection against SARS-CoV-2 infections is based on general effective principles, such as inhibition of pathogen adhesion and antimicrobial and immunomodulatory specific properties of different probiotic strains [118, 145, 148]. These mechanisms can enhance the elimination of the SARS-Cov-2 virus but also act preventively by suppressing bacterial coinfections that correlate with COVID-19 (Figure 2) [118, 148, 149].

During fermentation, probiotic strains produce specific bioactive peptides that block ACE-2 enzyme receptors, thus preventing the SARS-CoV-2 virus from binding to these active sites [4, 150, 151]. The remnants of dead probiotic cells can act as ACE-2 inhibitors as well. These bioactive peptides may modulate blood pressure due to the inhibition of the conversion of angiotensin-I to angiotensin-II. The possible effect of these peptides on reducing the progression of COVID-19 is still being examined [152]. The concept of using ACE-2 receptor-blocking drugs as a treatment modality for COVID-19 was first proposed by Fernandez-Fernandez [151]. Imai et al. [152] reported that the usage of ACE blockers had a positive effect on the reduction of respiratory distress syndrome. The study by Singh and Rao [25] confirmed that by binding mucosal cell receptor and ACE-2, probiotic strains interfere with coronavirus and block its binding, while an increase of innate immunity is stimulated by releasing intestinal mucins from mucosal cells and producing secretory antibodies (IgA). The authors stated that the key role in combati-

Table 1

Review of clinical studies which used probiotics in the prevention and therapy of COVID-19 disease *

Reference	Country	Study type	Subjects	Probiotic strain	Intervention	Main results
d'Ettorre et al. [135]	Italy	Single group	70 patients with COVID-19 hospitalized	*Streptococcus thermophilus* DSM 32345, *Lactobacillus acidophilus* DSM 32241, *Lactobacillus helveticus* DSM 32242, *Lacticaseibacillus paracasei* DSM 32243, *Lactiplantibacillus plantarum* DSM 32244, *Levilactobacillus brevis* DSM 27961, *Bifidobacterium lactis* DSM 32246, *Bifidobacterium lactis* DSM 32247	Daily oral 2.4 billion CFUs bacteria for a period of 14 days	Probiotic intervention demonstrated a significant improvement in clinical conditions among patients with COVID-19.
Tang et al. [136]	The United States	Double-blinded, randomized, placebo-controlled trial	1,132 individuals with household contacts who tested positive for COVID-19	*Lacticaseibacillus rhamnosus* GG (ATCC 53103)	Daily oral *Lacticaseibacillus rhamnosus* GG or placebo for a period of 28 days	Low-cost and safe probiotics can serve as a rapid intervention strategy in the prevention or reduction of symptoms of pandemic diseases.
Endam et al. [137]	Canada, Saudi Arabia, and the United States	Prospective randomized clinical trial	23 individuals between 18–59 years of age received late PCR positive tests for SARS-CoV-2	*Lactococcus lactis* W136	Nasal irrigations through a buffered isotonic solution containing 2.4×10^9 CFUs of *Lactococcus lactis* W136 or buffered isotonic saline isolated for two weeks (twice a day)	Probiotic intranasal intervention was correlated with a reduced number of patients showing moderate/severe symptoms of fatigue, loss of smell perception, and sensation of breathlessness, and by decreased percentage of individuals with moderate/severe facial pain or sore throat.
Gutierrez-Castrellon et al. [138]	Mexico and Spain	Single-center, quadruple-blinded randomized clinical trial	300 outpatients with symptomatic COVID-19 (ages 18–60) with positive nucleic acids test for SARS-CoV-2	*Lactiplantibacillus plantarum* KABP022, KABP023 and KAPB033, *Pediococcus acidilactici* KABP021	Daily ingestion of 10^9 CFUs for a period of 30 days	Remission was achieved by 53% of probiotic group compared to 28% in placebo group.
Mozota et al. [139]	Spain	Single group	29 residents of a nursing home who tested positive for COVID-19	*Ligilactobacillus salivarius* MP101	Daily consumption of 10^9 CFUs of *Ligilactobacillus salivarius* MP101 per unit of product (125 g)	Certain immune factors can be utilized as possible nasal or fecal biomarkers of benefits of probiotic strain supplementation in the diet of elderly people infected with SARS-CoV-2.
Wang et al. [21]	China	Retrospective study	138 patients	5×10^7 CFUs of live *Bifidobacterium longum*; live *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (should not be lower than 0.5×10^6 CFUs)	Four doses at a time, 3 times a day	Compared to the control group, patients treated with probiotics showed a significantly reduced time for achieving a negative nucleic acid test while the inflammation indexes, including PCT and CRP, were significantly reduced.
Wang et al. [140]	China	Randomized controlled clinical trial	200 frontline medical staff	One billion CFUs of *Streptococcus thermophilus* ENT-K12 over shelf-life	Slowly dissolving oral lozenge	Significantly reduced incidence of respiratory tract infections by 64.8%, reduced the time experiencing respiratory tract infections and oral ulcer symptoms

Table 1 (continued)

Wang et al. [140]	China	Randomized controlled clinical trial	200 frontline medical staff	One billion CFUs of *Streptococcus thermophilus* ENT-K12 over shelf-life	Slowly dissolving oral lozenge	Significantly reduced incidence of respiratory tract infections by 64.8%, reduced the time experiencing respiratory tract infections and oral ulcer symptoms by 78%, shortened the sick-leave days by 95.5%, and reduced the time under medication in cases when there was no record of antibiotic and anti-viral drug intake in the probiotic group.
Li et al. [141]	Wuhan, China	Retrospective single-center study	311 COVID-19 patients	*Lactobacillus rhamnosus* GG	Daily oral administration per 32 days	Moderates immunity and decreases the incidence of secondary infection in COVID-19 patients.
Bozkurt and Bilen [142]	Turkey	Retrospective study	44 moderate/severely ill adults	One trillion CFUs *Bifidobacterium* BB-12 strain	Oral administration in 250 mL water. Total doses were divided into three parts and administrated to patients for 3 days	Lower mortality, shortening the length of stay in hospital, early radiologic improvement and decrease plasma IL-6 level in moderate/severe SARS-CoV-2 patients in the probiotic group.
Wischmeyer et al. [143]	The United States	Randomized, double-blind, placebo-controlled trial	182 participants	*Lactobacillus rhamnosus* GG	Daily oral use for 28 days	Prolonged time for development of COVID-19 infection, reduced incidence of symptoms and changes to gut microbiome structure when used as post-exposure prophylaxis within seven days after probiotic exposure.

COVID-19 – corona virus disease-19; CFUs – colony forming units; PCR – polymerase chain reaction test; CRP – c-reactive protein; PCT – procalcitonin; IL – interleukin; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2.
***Modified Table of Xavier-Santos et al. [134]**

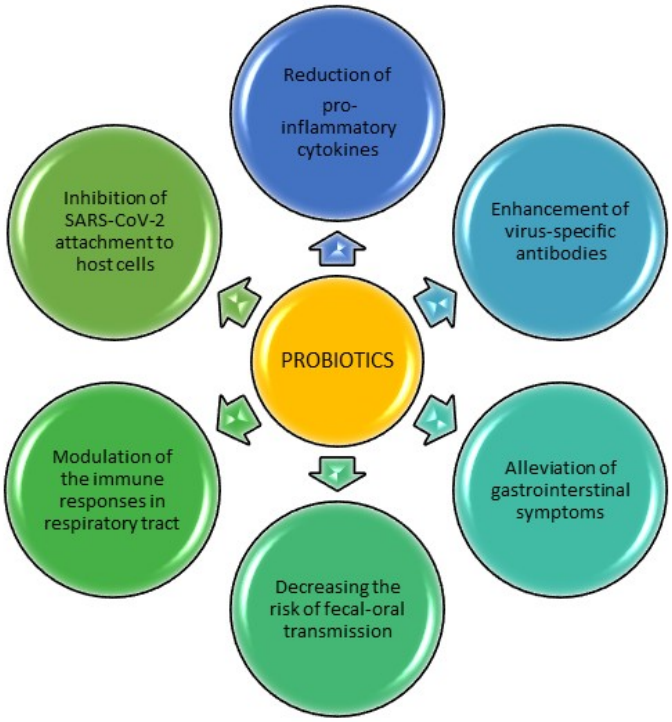

Fig. 2 - Antiviral mechanisms of probiotics against SARS-CoV-2 infection.

ng coronavirus proliferation is played by modulation of immune responses and balance of acquired immunity, production of inflammatory cytokines, the proliferation of B cells that produce specific antibodies, and activation of cytotoxic T lymphocytes that participate in the adaptive immune response [27]. Baindara et al. [126] also showed that some probiotics improved the regulatory activity of T cells and reduced the production of proinflammatory cytokines. In addition, the antiviral effect of probiotics may be achieved by a large number of secreted specific metabolites and bacteriocins [153].

Strengthening the immune response during incubation and the initial phase of COVID-19 disease is crucial in eliminating the virus and preventing the progression of the disease. The use of certain strains of *Bifidobacterium* or *Lactobacillus* has a great influence on the elimination of the SARS-CoV-2 virus from respiratory organs [154]. The use of probiotics, along with adequate treatment for COVID-19, can significantly reduce the occurrence and duration of various systemic diseases [154, 155].

The limitations of this comprehensive literature review arise due to the high heterogeneity of studies that investigated the change and impact of oral microbiota during COVID-19 without knowing the previous status of patients' OM and because patients with different immune statuses used different probiotic strains.

It should be emphasized that introducing targeted drugs and beneficial bacteria was of great importance in restoring the damaged microbiome. Further research should be directed to discovering the most effective probiotic strains, doses, and formulations, as well as the interaction of probiotics and microbiomes. In addition, the influence of environmental factors on the oropharyngeal microbiome, as well as possible coinfection, should be further investigated.

Conclusion

After an extensive review of the literature, it was concluded that numerous clinical studies showed that OM might influence resistance to primary infection and be a predictor for disease severity and complications during COVID-19. The use of probiotic strains can inhibit the adhesion of pathogens, improve the barrier function of the intestine and strengthen the immune response. Through these mechanisms, probiotics can reduce the progression and the development of more severe forms of the disease, shorten the hospital stay and reduce the frequency of post-COVID syndrome.

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R E F E R E N C E S

1. World health organization (WHO). WHO coronavirus (COVID-19) dashboard. [Internet] 2022. Available from: https://covid19.who.int/ [Accessed on 22 Apr 2022].
2. *Ye ZW, Yuan S, Yuen KS, Fung SY, Chan CP, Jin DY*. Zoonotic origins of human coronaviruses. Int J Biol Sci 2020; 16(10): 1686–97.
3. *Łoniewski I, Skonieczna-Żydecka K, Sołek-Pastuszka J, Marlicz W*. Probiotics in the management of mental and gastrointestinal post-COVID symptomes. J Clin Med 2022; 11(17): 5155.
4. *Baghbani T, Nikzad H, Azadbakht J, Izadpanah F, Haddad Kashani H*. Dual and mutual interaction between microbiota and viral infections: a possible treat for COVID-19. Microb Cell Fact 2020; 19(1): 217.
5. *Soffritti I, D'Accolti M, Fabbri C, Passaro A, Manfredini R, Zuliani G*, et al. Oral mycrobiome dysbiosis is associated with symptoms severity and local immune/inflammatory response in COVID-19 patients: a cross-sectional study. Front Microbiol 2021; 12: 687513.
6. *Bohórquez-Ávila S, Bernal-Cepeda L, Reina-Marin M, Navarro-Saiz L, Castellanos J*. The mouth, oral health, and infection with SARS-CoV-2: an underestimated topic. Infectio 2022; 26 (1): 78–82.
7. *To KK, Tsang OT, Yip CC, Chan KH, Wu TC, Chan JM*, et al. Consistent detection of 2019 novel coronavirus in saliva. Clin Infect Dis 2020; 71(15): 841–3.
8. *Amorim Dos Santos J, Normando AGC, Carvalho da Silva RL, De Paula RM, Cembranel AC, Santos-Silva AR*, et al. Oral mucosal lesions in a COVID-19 patient: new signs or secondary manifestations? Int J Infect Dis 2020; 97: 326–8.
9. *Bao L, Zhang C, Dong J, Zhao L, Li Y, Sun J*. Oral mycrobiome and SARS-CoV-2: beware of lung co-infection. Front Microbiol 2020; 11: 1840.
10. *Silva A, Azevedo M, Sampaio-Maia B, Sousa-Pinto B*. The effect of mouthrinses on severe acute respiratory syndrome coronavirus 2 viral load: a systematic review. J Am Dent Assoc 2022: 635–48. [Epub ahead of print].
11. *Paradowska-Stolarz AM*. Oral manifestations of COVID-19: brief review. Dent Med Probl 2021; 58(1): 123–6.
12. *Xu J, Li Y, Gan F, Du Y, Yao Y*. Salivary glands: Potential reservoirs for COVID-19 asymptomatic infection. J Dent Res 2020; 99(8): 989.
13. *Mohapatra RK, Dhama K, Mishra S, Sarangi AK, Kandi V, Tiwari R*, et al. The microbiota-related coinfections in COVID-19 patients: a real challenge. Beni Suef Univ J Basic Appl Sci 2021;10 (1): 47.
14. *Rocchi G, Giovanetti M, Benedetti F, Borsetti A, Ceccarelli G, Zella D*, et al. Gut microbiota and COVID-19: potential implications for disease severity. Pathogens 2022; 11(9): 1050.
15. *Amrouche T, Chikindas ML*. Probiotics for immunomodulation in prevention against respiratory viral infections with special emphasis on COVID-19. AIMS Microbiol 2022; 8(3): 338–56.
16. *Cyprian F, Sohail MU, Abdelhafez I, Salman S, Attique Z, Kamareddine L*, et al. SARS-CoV-2 and immune-microbiome interactions: lessons from respiratory viral infections. Int J Infect Dis 2021; 105: 540–50.
17. *Rafiqul Islam SM, Foysal MJ, Hoque MN, Mehedi HMH, Rob MA, Salauddin A,* et al. Dysbiosis of oral and gut microbiomes in SARS-CoV-2 infected patients in Bangladesh: elucidating the role of opportunistic gut microbes. Front Med (Lausanne) 2022; 9: 821777.
18. *Herrera D, Serrano J, Roldán S, Sanz M*. Is the oral cavity relevant in SARS-CoV-2 pandemic? Clin Oral Investig 2020; 24(8): 2925–30.
19. *Zhao H, Chen S, Yang F, Wu H, Ba Y, Cui L*, et al. Alternation of nasopharyngeal microbiota in healthy youth is associated with environmental factors: implication for respiratory diseases. Int J Environ Health Res 2022; 32(5): 952–62.
20. *Iebba V, Zanotta N, Campisciano G, Zerbato V, Di Bella S, Cason C,* et al. Profiling of oral microbiota and cytokines in COVID-19 patients. Front Microbiol 2021; 12: 671813.
21. *Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J,* et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 2020; 323(11): 1061–9.
22. *Barros MIOS, Firmino GLO, Vieira T da S, Araujo A de A, Souza L de LB, Reis BS,* et al. Oral manifestations associated with COVID-19 infection. RSD [Internet] 2021; [accessed on 19 Mar 2023] 10(16): e555101624107. Available from: https://rsdjournal.org/index.php/rsd/article/view/24107
23. *Haran JP, Bradley E, Zeamer AL, Cincotta L, Salive MC, Dutta P*, et al. Inflammation-type dysbiosis of the oral mycrobiome associates with the duration of COVID-19 symptoms and long COVID. JCI Insight 2021; 6(20): e152346.
24. *Ward DV, Bhattarai S, Rojas-Correa M, Purkayastha A, Holler D, Qu MD,* et al. The intestinal and oral microbiomes are robust predictors of COVID-19 severity the main predictor of COVID-19-related fatality [preprint]. 2021; medRxiv: 2021.01.05.20249061. Available from: https://doi.org/doi:10.1101/2021.01.05.20249061
25. *Singh K, Rao A*. Probiotics: a potential immunomodulator in COVID-19 infection management. Nutr Res 2021; 87: 1–12.
26. *Ursell LK, Metcalf JL, Parfrey LW, Knight R*. Defining the human microbiome. Nutr Rev 2012; 70 (Suppl 1): S38–44.
27. *Santacroce L, Charitos IA, Ballini A, Inchingolo F, Luperto P, De Nitto E,* et al. The human respiratory system and its microbiome at a glimpse. Biology (Basel) 2020; 9(10): 318.
28. *Santacroce L, Sardaro N, Topi S, Pettini F, Bottalico L, Cantore S,* et al. The pivotal role of oral microbiota in health and disease. J Biol Regul Homeost Agents 2020; 34(2): 733–7.
29. *Gomez PAM*. Use of probiotics in dentistry. Dent Oral Craniofac Res 2017; 4(1): 1–4.
30. *Lamont RJ, Koo H, Hajishengallis G*. The oral microbiota: dynamic communities and host interactions. Nat Rev Microbiol 2018; 16(12): 745–59.
31. *Caselli E, Fabbri C, D'Accolti M, Soffritti I, Bassi C, Mazzacane S*, et al. Defining the oral mycrobiome by whole-genome sequencing and resistome analysis: the complexity of the healthy picture. BMC Microbiol 2020; 20(1): 120.
32. *Radaic A, Kapila YL*. The oralome and its dysbiosis: new insights into oral microbiome-host interactions. Comput Struct Biotechnol J 2021; 19: 1335–60.
33. *Wade WG*. The oral mycrobiome in health and disease. Pharmacol Res 2013; 69(1): 137–43.
34. *Rowan-Nash AD, Korry BJ, Mylonakis E, Belenky P*. Cross-domain and viral interactions in the microbiome. Microbiol Mol Biol Rev 2019; 83(1): e00044–18.
35. *Yamamoto S, Saito M, Tamura A, Prawisuda D, Mizutani T, Yotsuyanagi H*. The human microbiome and COVID-19: a systematic review. PLoS One 2021; 16(6): e0253293.
36. *Biagi E, Candela M, Fairweather-Tait S, Franceschi C, Brigidi P*. Aging of the human metaorganism: the microbial counterpart. Age (Dordr) 2012; 34(1): 247–67.
37. *Park SH, Kim KA, Ahn YT, Jeong JJ, Huh CS, Kim DH*. Comparative analysis of gut microbiota in elderly people of urbanized towns and longevity villages. BMC Microbiol 2015; 15: 49.
38. *Arweiler NB, Netuschil L*. The oral microbiota. Adv Exp Med Biol 2016; 902: 45–60.
39. *Brooks AW, Priya S, Blekhman R, Bordenstein SR*. Gut microbiota diversity across ethnicities in the United States. PLoS Biol 2018; 16(12): e2006842.

40. *Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A*, et al. What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. Microorganisms 2019; 7(1): 14.
41. *Liu J, Lahousse L, Nivard MG, Bot M, Chen L, van Klinken JB*, et al. Integration of epidemiologic, pharmacologic, genetic and gut microbiome data in a drug-metabolite atlas. Nat Med 2020; 26(1): 110–7.
42. *Boyajian JL, Ghebretatios M, Schaly S, Islam P, Prakash S*. Microbiome and human aging: probiotic and prebiotic potentials in longevity, skin health and cellular senescence. Nutrients 2021; 13(12): 4550.
43. *Pérez-Brocal V, Moya A*. The analysis of the oral DNA virome reveals which viruses are widespread and rare among healthy young adults in Valencia (Spain). PLoS One 2018; 13(2): e0191867.
44. *Baker JL, Bor B, Agnello M, Shi W, He X*. Ecology of the oral microbiome: beyond bacteria. Trends Microbiol 2017; 25(5): 362–74.
45. de la Cruz Peña MJ, Martinez-Hernandez F, Garcia-Heredia I, Lluesma Gomez M, Fornas Ò, Martinez-Garcia M. Deciphering the human virome with single-virus genomics and metagenomics. Viruses 2018; 10(3): 113.
46. *Peters BA, Wu J, Hayes RB, Ahn J*. The oral fungal mycobiome: characteristics and relation to periodontitis in a pilot study. BMC Microbiol 2017; 17(1): 157.
47. *Mosaddad SA, Tahmasebi E, Yazdanian A, Rezvani MB, Seifalian A, Yazdanian M*, et al. Oral microbial biofilms: an update. Eur J Clin Microbiol Infect Dis 2019; 38(11): 2005–19.
48. *Dubar M, Zaffino ML, Remen T, Thilly N, Cunat L, Machouart MC*, et al. Protozoans in subgingival biofilm: clinical and bacterial associated factors and impact of scaling and root planing treatment. J Oral Microbiol 2019; 12(1): 1693222.
49. *Belmok A, de Cena JA, Kyaw CM, Damé-Teixeira N*. The oral archaeome: a scoping review. J Dent Res 2020; 99(6): 630–43.
50. *Lu M, Xuan S, Wang Z*. Oral microbiota: A new view of body health. Food Sci Hum Wellness 2019; 8(1): 8–15.
51. *Stubbendieck RM, May DS, Chevrette MG, Temkin MI, Wendt-Pienkowski E, Cagnazzo J,* et al. Competition among nasal bacteria suggests a role for siderophore-mediated interactions in shaping the human nasal microbiota. Appl Environ Microbiol 2019; 85(10): e02406–18.
52. *Gebrayel P, Nicco C, Al Khodor S, Bilinski J, Caselli E, Comelli EM,* et al. Microbiota medicine: towards clinical revolution. J Transl Med 2022; 20(1): 111.
53. *Edwards V, Smith DL, Meylan F, Tiffany L, Poncet S, Wu WW,* et al. Analyzing the role of gut microbiota on the onset of autoimmune diseases using $TNF^{\Delta ARE}$ murine model. Microorganisms 2021; 10(1): 73.
54. *Petersen C, Round JL*. Defining dysbiosis and its influence on host immunity and disease. Cell Microbiol 2014; 16(7): 1024–33.
55. *Nikolić-Jakoba N, Vojnović S, Pavić A, Janković S, Leković V, Vasiljević B*. Polymerase chain reaction in the identification of periodontopathogens: a reliable and satisfactory method? Arch Biol Sci 2012; 64(4): 1413–23.
56. *Tandelilin RT, Widita E, Agustina D, Saini R*. The effect of oral probiotic consumption on the caries risk factors among high-risk caries population. J Int Oral Health 2018; 10(3): 132–7.
57. *Zaura E, Twetman S*. Critical appraisal of oral pre- and probiotics for caries prevention and care. Caries Res 2019; 53(5): 514–26. https://doi.org/10.1159/000499037. PMid: 30947169
58. *Ayala LDO, Zambrano JFB, Vire JMY, Gavilanes MPP, Coyago M de LR*. Modulation of oral biofilm and immune response associated to mucosa with probiotic bacteria as a potential approach in the prevention of dental caries: a systematic review. Dent Oral Biol Craniofac Res 2020; 3(5): 1–7.
59. *Talaat D*. Effect of probiotic chewable tablets on oral health and white spot lesions in pre-school children: a randomized clinical trial. Egypt Dent J 2021; 67(3): 1797–807.
60. *Milićević R, Brajović G, Nikolić-Jakoba N, Popović B, Pavlica D, Leković V,* et al. Identification of periodontopathogen microorganisms by PCR technique. Srp Arh Celok Lek 2008; 136(9–10): 476–80. (Serbian)
61. *Kumar PS*. From focal sepsis to periodontal medicine: a century of exploring the role of the oral mycrobiome in systemic disease. J Physiol 2017; 595(2): 465–76.
62. *Allaker RP, Stephen AS*. Use of probiotics and oral health. Curr Oral Health Rep 2017; 4(4): 309–18.
63. *Radović N, Nikolić-Jakoba N, Petrović N, Milosavljević A, Brković B, Roganović J*. MicroRNA-146a and microRNA-155 as novel crevicular fluid biomarkers for periodontitis in nondiabetic and type 2 diabetic patients. J Clin Periodontol 2018; 45(6): 663–71.
64. *Ratna Sudha M, Neelamraju J, Surendra Reddy M, Kumar M*. Evaluation of the effect of probiotic *Bacillus coagulans* unique IS2 on mutans Streptococci and Lactobacilli levels in saliva and plaque: a double-blind, randomized, placebo-controlled study in children. Int J Dent 2020; 2020: 8891708.
65. *Wang L, Ganly I*. The oral mycrobiome and oral cancer. Clin Lab Med 2014; 34(4): 711–9.
66. *Li Y, He J, He Z, Zhou Y, Yuan M, Xu X*, et al. Phylogenetic and functional gene structure shifts of the oral microbiomes in periodontitis patients. ISME J 2014; 8(9): 1879–91.
67. *Abusleme L, Morandini AC, Hashizume-Takizawa T, Sahingur SE*. Editorial: Oral mycrobiome and inflammation connection to systemic health. Front Cell Infect Microbiol 2021; 11: 780182.
68. *Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F*, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature 2012; 490(7418): 55–60.
69. *Marchi-Alves LM, Freitas D, de Andrade D, de Godoy S, Toneti AN, Mendes IAC*. Characterization of oral microbiota in removable dental prosthesis users: influence of arterial hypertension. Biomed Res Int 2017; 2017: 3838640.
70. *Bryan NS, Tribble G, Angelov N*. Oral mycrobiome and nitric oxide: the missing link in the management of blood pressure. Curr Hypertens Rep 2017; 19(4): 33.
71. *Cobb CM, Kelly PJ, Williams KB, Babbar S, Angolkar M, Derman RJ*. The oral mycrobiome and adverse pregnancy outcomes. Int J Womens Health 2017; 9: 551–9.
72. *Chen C, Hemme C, Beleno J, Shi ZJ, Ning D, Qin Y*, et al. Oral microbiota of periodontal health and disease and their changes after nonsurgical periodontal therapy. ISME J 2018; 12(5): 1210–24.
73. *Kaul D, Rathnasinghe R, Ferres M, Tan GS, Barrera A, Pickett BE*, et al. Microbiome disturbance and resilience dynamics of the upper respiratory tract during influenza A virus infection. Nat Commun 2020; 11(1): 2537.
74. *Lee HS, Lobbestael E, Vermeire S, Sabino J, Cleynen I*. Inflammatory bowel disease and Parkinson's disease: common pathophysiological links. Gut 2021; 70(2): 408–17.
75. *Willmott T, McBain AJ, Humphreys GJ, Myers J, Cottrell E*. Does the oral mycrobiome play a role in hypertensive pregnancies? Front Cell Infect Microbiol 2020; 10: 389.
76. *Sohail MU, Hedin L, Al-Asmakh M*. Dysbiosis of the salivary microbiome is associated with hypertension and correlated with metabolic syndrome biomarkers. Diabetes Metab Syndr Obes 2021; 14: 4641–53.
77. *Milasin J, Nikolić-Jakoba N, Stefanović D, Sopta J, Pucar A, Leković V*, et al. Periodontal inflammation as risk factor for pancreatic diseases. In: *Nagal A*, editor. Inflammatory diseases - a modern perspective. London: IntechOpen; 2011.
78. *Đorđević V, Jovanović M, Stefanović V, Nikolić Jakoba N, Đokić G, Stašević Karličić I*, et al. Assessment of periodontal health among

the inpatients with schizophrenia. Vojnosanit Pregl 2019; 76(11): 1139–46.
79. *Ren Z, Wang H, Cui G, Lu H, Wang L, Luo H*, et al. Alterations in the human oral and gut microbiomes and lipidomics in COVID-19. Gut 2021; 70(7): 1253–65.
80. *Zhang X, Zhang D, Jia H, Feng Q, Wang D, Liang D*, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med 2015; 21(8): 895–905.
81. *Kurt BS, Ilhan B, Sevki BI, Kurt AF, Orhan K.* Periodontal management during COVID-19 pandemic: mini review. Balk J Dent Med 2021; 25(3): 135–8.
82. *Rakić M, Nikolić-Jakoba N, Struillout X, Petković-Ćurčin A, Stamatović N, Matić S*, et al. Receptor activator of nuclear factor kappa B (RANK) as a determinant of peri-implantitis. Vojnosanit Pregl 2013; 70(4): 346–51.
83. *Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM*, et al. The oral mycrobiome - an update for oral healthcare professionals. Br Dent J 2016; 221(10): 657–66.
84. *Belkaid Y, Hand TW*. Role of the microbiota in immunity and inflammation. Cell 2014; 157(1): 121–41.
85. *Trompette A, Gollwitzer ES, Pattaroni C, Lopez-Mejia IC, Riva E, Pernot J*, et al. Dietary fiber confers protection against flu by shaping Ly6c- patrolling monocyte hematopoiesis and CD8+ T cell metabolism. Immunity 2018; 48(5): 992–1005. e8.
86. *Willis JR, Gabaldón T*. The human oral mycrobiome in health and disease: from sequences to ecosystems. Microorganisms 2020; 8(2): 308.
87. *Lynch SV*. Viruses and microbiome alterations. Ann Am Thorac Soc 2014; 11(Suppl 1): S57–60.
88. *Li N, Ma WT, Pang M, Fan QL, Hua JL*. The commensal microbiota and viral infection: a comprehensive review. Front Immunol 2019; 10: 1551.
89. *Scannapieco FA*. Role of oral bacteria in respiratory infection. J Periodontol 1999; 70(7): 793–802.
90. *Khan R, Petersen FC, Shekhar S*. Commensal Bacteria: An emerging player in defense against respiratory pathogens. Front Immunol 2019; 10: 1203.
91. *Pfeiffer JK, Sonnenburg JL*. The intestinal microbiota and viral susceptibility. Front Microbiol 2011; 2: 92.
92. *Hanada S, Pirzadeh M, Carver KY, Deng JC*. Respiratory viral infection-induced microbiome alterations and secondary bacterial pneumonia. Front Immunol 2018; 9: 2640.
93. *Ai JW, Zhang HC, Xu T, Wu J, Zhu M, Yu YQ*, et al. Optimizing diagnostic strategy for novel coronavirus pneumonia, a multi-center study in Eastern China [preprint]. 2020; medRxiv: 2020.02.13.20022673. Available from: https://www.medrxiv.org/content/10.1101/2020.02.13.20022673v1
94. *Xu K, Cai H, Shen Y, Ni Q, Chen Y, Hu S*, et al. Management of COVID-19: the Zhejiang experience. Zhejiang Da Xue Xue Bao Yi Xue Ban 2020; 49(2): 147–57. (Chinese)
95. *Cox MJ, Loman N, Bogaert D, O'Grady J*. Co-infections: potentially lethal and unexplored in COVID-19. Lancet Microbe 2020; 1(1): e11.
96. *Roncati L, Lusenti B, Nasillo V, Manenti A*. Fatal SARS-CoV-2 coinfection in course of EBV-associated lymphoproliferative disease. Ann Hematol 2020; 99(8):1945–6.
97. *Chakraborty S*. Metagenome of SARS-Cov-2 patients in Shenzhen with travel to Wuhan shows a wide range of species - Lautropia, Cutibacterium, Haemophilus being most abundant - and Campylobacter explaining diarrhea [preprint]. OSF Preprints 2020; doi: 10.31219/osf.io/jegwq.
98. *Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG*, et al. A new coronavirus associated with human respiratory disease in China. Nature 2020; 579(7798): 265–9. Erratum in: Nature 2020; 580(7803): E7.
99. *Chen T, Song J, Liu H, Zheng H, Chen C*. Positive Epstein-Barr virus detection in coronavirus disease 2019 (COVID-19) patients. Sci Rep 2021; 11(1): 10902.
100. *Yeoh YK, Zuo T, Lui GC, Zhang F, Liu Q, Li AY*, et al. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. Gut 2021; 70(4): 698–706.
101. *Marouf N, Cai W, Said KN, Daas H, Diab H, Chinta VR*, et al. Association between periodontitis and severity of COVID-19 infection: a case-control study. J Clin Periodontol 2021; 48(4): 483–91.
102. *Song WJ, Hui CKM, Hull JH, Birring SS, McGarvey L, Mazzone SB*, et al. Confronting COVID-19-associated cough and the post-COVID syndrome: role of viral neurotropism, neuroinflammation, and neuroimmune responses. Lancet Respir Med 2021; 9(5): 533–44.
103. *Oronsky B, Larson C, Hammond TC, Oronsky A, Kesari S, Lybeck M*, et al. A review of persistent post-COVID syndrome (PPCS). Clin Rev Allergy Immunol 2023; 64(1): 66–74; Epub 2021; 20: 1–9.
104. *France K, Glick M*. Long COVID and oral health care considerations. J Am Dent Assoc 2022; 153(2): 167–74.
105. *Larsen JM*. The immune response to Prevotella bacteria in chronic inflammatory disease. Immunology 2017; 151(4): 363–74.
106. *Xu L, Chen X, Wang Y, Jiang W, Wang S, Ling Z*, et al. Dynamic alterations in salivary microbiota related to dental caries and age in preschool children with deciduous dentition: a 2-year follow-up study. Front Physiol 2018; 9: 342.
107. *Khan AA, Khan Z*. COVID-2019-associated overexpressed Prevotella proteins mediated host-pathogen interactions and their role in coronavirus outbreak. Bioinformatics 2020; 36(13): 4065–9.
108. *Wu Y, Cheng X, Jiang G, Tang H, Ming S, Tang L*, et al. Author correction: Altered oral and gut microbiota and its association with SARS-CoV-2 viral load in patients with COVID-19 during hospitalization. NPJ Biofilms Microbiomes 2021; 7(1): 90. Erratum for: NPJ Biofilms Microbiomes 2021; 7(1): 61.
109. *Jasinski-Bergner S, Mandelboim O, Seliger B*. Molecular mechanisms of human herpes viruses inferring with host immune surveillance. J Immunother Cancer 2020; 8(2): e000841.
110. *Azarpazhooh A, Leake JL*. Systematic review of the association between respiratory diseases and oral health. J Periodontol 2006; 77(9): 1465–82.
111. *Hols P, Ledesma-García L, Gabant P, Mignolet J*. Mobilization of microbiota commensals and their bacteriocins for therapeutics. Trends Microbiol 2019; 27(8): 690–702.
112. *Barbour A, Wescombe P, Smith L*. Evolution of Lantibiotic Salivaricins: New weapons to fight infectious diseases. Trends Microbiol 2020; 28(7): 578–93.
113. *Hadžić Z, Pašić E, Gojkov-Vukelić M, Hadžić S*. Effects of Lactobacillus reuteri lozenges (Prodentis) as adjunctive therapeutic agent in non-surgical therapy of periodontitis. Balk J Dent Med 2021; 25(1): 41–5.
114. *Bottari B, Castellone V, Neviani E*. Probiotics and Covid-19. Int J Food Sci Nutr 2021; 72(3): 293–9.
115. *Mikulicic A, Bakarcic D, Ivancic Jokic N, Hrvatin S, Culav T*. The use of probiotics in dental medicine. Madridge J Dent Oral Surg 2017; 2(1): 44–6.
116. *Bermudez-Brito M, Plaza-Díaz J, Muñoz-Quezada S, Gómez-Llorente C, Gil A*. Probiotic mechanisms of action. Ann Nutr Metab 2012; 61(2): 160–74.
117. *Eguchi K, Fujitani N, Nakagawa H, Miyazaki T*. Prevention of respiratory syncytial virus infection with probiotic lactic acid

bacterium Lactobacillus gasseri SBT2055. Sci Rep 2019; 9(1): 4812.

118. *Morawvej H, Memariani H, Memariani M*. Therapeutic and preventive potential of probiotics against COVID-19. Res Bull Med Sci 2020; 25(1): e18.
119. *Azad MAK, Sarker M, Wan D*. Immunomodulatory effects of probiotics on cytokine profiles. Biomed Res Int 2018; 2018: 8063647.
120. *Hardy H, Harris J, Lyon E, Beal J, Foey AD*. Probiotics, prebiotics and immunomodulation of gut mucosal defences: homeostasis and immunopathology. Nutrients 2013; 5(6): 1869–912.
121. *Al Kassaa I*. The antiviral activity of probiotic metabolites. In: *Al Kassaa I*. New insights on antiviral probiotics. Springer, Cham: 2017; 83–97.
122. *Xia Y, Cao J, Wang M, Lu M, Chen G, Gao F,* et al. Effects of Lactococcus lactis subsp. lactis JCM5805 on colonization dynamics of gut microbiota and regulation of immunity in early ontogenetic stages of tilapia. Fish Shellfish Immunol 2019; 86: 53–63.
123. *Hung YP, Lee CC, Lee JC, Tsai PJ, Ko WC*. Gut dysbiosis during COVID-19 and potential effect of probiotics. Microorganisms 2021; 9(8): 1605.
124. *Al Kassaa I, Hober D, Hamze M, Chihib NE, Drider D*. Antiviral potential of lactic acid bacteria and their bacteriocins. Probiotics Antimicrob Proteins 2014; 6(3–4): 177–85.
125. *Mahooti M, Abdolalipour E, Salehzadeh A, Mohebbi SR, Gorji A, Ghaemi A*. Immunomodulatory and prophylactic effects of Bifidobacterium bifidum probiotic strain on influenza infection in mice. World J Microbiol Biotechnol 2019; 35(6): 91.
126. *Baindara P, Chakraborty R, Holliday ZM, Mandal SM, Schrum AG*. Oral probiotics in coronavirus disease 2019: connecting the gut-lung axis to viral pathogenesis, inflammation, secondary infection and clinical trials. New Microbes New Infect 2021; 40: 100837.
127. *Małaczewska J, Kaczorek-Łukowska E, Wójcik R, Siwicki AK*. Antiviral effects of nisin, lysozyme, lactoferrin and their mixtures against bovine viral diarrhoea virus. BMC Vet Res 2019; 15(1): 318.
128. *Di Pierro F*. A possible probiotic (S. salivarius K12) approach to improve oral and lung microbiotas and raise defenses against SARS-CoV-2. Minerva Med 2020; 111(3): 281–3.
129. *Zhang H, Yeh C, Jin Z, Ding L, Liu BY, Zhang L*, et al. Prospective study of probiotic supplementation results in immune stimulation and improvement of upper respiratory infection rate. Synth Syst Biotechnol 2018; 3(2): 113–20.
130. *Seminario-Amez M, López-López J, Estrugo-Devesa A, Ayuso-Montero R, Jané-Salas E*. Probiotics and oral health: a systematic review. Med Oral Patol Oral Cir Bucal 2017; 22(3): e282–8.
131. *Conte L, Toraldo DM*. Targeting the gut-lung microbiota axis by means of a high-fibre diet and probiotics may have anti-inflammatory effects in COVID-19 infection. Ther Adv Respir Dis 2020; 14: 1753466620937170.
132. *Olaimat AN, Aolymat I, Al-Holy M, Ayyash M, Abu Ghoush M, Al-Nabulsi AA*, et al. The potential application of probiotics and prebiotics for the prevention and treatment of COVID-19. NPJ Sci Food 2020; 4: 17.
133. *Stavropoulou E, Bezirtzoglou E*. Probiotics in medicine: a long debate. Front Immunol 2020; 11: 2192.
134. *Xavier-Santos D, Padilha M, Fabiano GA, Vinderola G, Gomes Cruz A, Sivieri K*, et al. Evidences and perspectives of the use of probiotics, prebiotics, synbiotics, and postbiotics as adjuvants for prevention and treatment of COVID-19: a bibliometric analysis and systematic review. Trends Food Sci Technol 2022; 120: 174–92. Erratum in: Trends Food Sci Technol 2022; 121: 156–160.
135. *d'Ettorre G, Ceccarelli G, Marazzato M, Campagna G, Pinacchio C, Alessandri F*, et al. Challenges in the management of SARS-CoV2 infection: the role of oral bacteriotherapy as complementary therapeutic strategy to avoid the progression of COVID-19. Front Med (Lausanne) 2020; 7: 389.
136. *Tang H, Bohannon L, Lew M, Jensen D, Jung SH, Zhao A*, et al. Randomised, double-blind, placebo-controlled trial of probiotics to eliminate COVID-19 transmission in exposed household contacts (PROTECT-EHC): a clinical trial protocol. BMJ Open 2021; 11(5): e047069.
137. *Endam LM, Tremblay C, Filali A, Desrosiers MY*. Intranasal application of *Lactococcus lactis* W136 bacteria early in SARS-CoV-2 infection may have a beneficial immunomodulatory effect: A proof-of-concept study [preprint]. 2021; medRxiv: 2021.01.05.20249061. Available from: https://www.medrxiv.org/content/10.1101/2021.04.18.21255699v1.full
138. *Gutierrez-Castrellon P, Gandara-Martí T, Abreu AT, Nieto-Rufino CD, Lopez- Orduna E, Jimenez-Escobar I*, et al. Efficacy and safety of novel probiotic formulation in adult Covid19 outpatients: a randomized, placebo-controlled clinical trial [preprint]. 2021; medRxiv: 2021.05.20.21256954. Available from: https://www.medrxiv.org/content/10.1101/2021.05.20.21256954v1.full.pdf+html
139. *Mozota M, Castro I, Gomez-Torres N, Arroyo R, Lailla Y, Somada M,* et al. Administration of *Ligilactobacillus salivarius* MP101 in an elderly nursing home during the COVID-19 pandemic: immunological and nutritional impact. Foods 2021; 10(9): 2149.
140. *Wang Q, Lin X, Xiang X, Liu W, Fang Y, Chen H*, et al. Oropharyngeal probiotic ENT-K12 prevents respiratory tract infections among frontline medical staff fighting against COVID-19: a pilot study. Front Bioeng Biotechnol 2021; 9: 646184.
141. *Li M, He Z, Yang J, Guo Q, Weng H, Luo J*, et al. Clinical characteristics, outcomes, and risk factors of disease severity in patients with COVID-19 and with a history of cerebrovascular disease in Wuhan, China: A Retrospective Study. Front Neurol 2022; 12: 706478.
142. *Bozkurt HS, Bilen Ö*. Oral booster probiotic bifidobacteria in SARS-COV-2 patients. Int J Immunopathol Pharmacol 2021; 35: 20587384211059677.
143. *Wischmeyer PE, Tang H, Ren Y, Bohannon L, Ramirez ZE, Andermann TM,* et al. Daily lactobacillus probiotic versus placebo in COVID-19-exposed household contacts (PROTECT-EHC): a randomized clinical trial [preprint]. 2022; medRxiv: 2022.01.04.21268275. Available from: https://www.medrxiv.org/content/10.1101/2022.01.04.21268275v1
144. *Block J*. High risk COVID-19: potential intervention at multiple points in the COVID-19 disease process via prophylactic treatment with azithromycin or bee derived products. J Biomed Res Rev 2020; 3(1): 26–31.
145. *Horowitz RI, Freeman PR, Bruzzese J*. Efficacy of glutathione therapy in relieving dyspnea associated with COVID-19 pneumonia: a report of 2 cases. Respir Med Case Rep 2020; 30: 101063.
146. *Pourhossein M, Moravejolahkami AR*. Probiotics in viral infections, with a focus on COVID-19: a systematic review [preprint]. Authorea 2020; doi: 10.22541/au.158999387.76467979.
147. *Ceccarelli G, Borrazzo C, Pinacchio C, Santinelli L, Innocenti GP, Cavallari EN*, et al. Oral bacteriotherapy in patients with COVID-19: a retrospective cohort study. Front Nutr 2021; 7: 613928.
148. *Patra S, Saxena S, Sahu N, Pradhan B, Roychowdhury A*. Systematic network and meta-analysis on the antiviral mechanisms of probiotics: a preventive and treatment strategy to mitigate SARS-CoV-2 infection. Probiotics Antimicrob Proteins 2021;13(4): 1138–56.

149. *Ayyash M, Olaimat A, Al-Nabulsi A, Liu SQ*. Bioactive properties of novel probiotic *Lactococcus lactis* fermented camel sausages: cytotoxicity, angiotensin converting enzyme inhibition, antioxidant capacity, and antidiabetic activity. Food Sci Anim Resour 2020; 40(2): 155–71.

150. *Nayebi A, Navashenaq JG, Soleimani D, Nachvak SM*. Probiotic supplementation: a prospective approach in the treatment of COVID-19. Nutr Health 2022; 28(2): 163–75.

151. *Fernández-Fernández FJ*. COVID-19, hypertension and angiotensin receptor-blocking drugs. J Hypertens 2020; 38(6): 1191.

152. *Imai Y, Kuba K, Rao S, Huan Y, Guo F, Guan B*, et al. Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 2005; 436(7047): 112–6.

153. *Spagnolello O, Pinacchio C, Santinelli L, Vassalini P, Innocenti GP, De Girolamo G,* et al. Targeting microbiome: an alternative strategy for fighting SARS-CoV-2 infection. Chemotherapy 2021; 66(1–2): 24–32.

154. *Infusino F, Marazzato M, Mancone M, Fedele F, Mastroianni CM, Severino P,* et al. Diet supplementation, probiotics, and nutraceuticals in SARS-CoV-2 infection: a scoping review. Nutrients 2020; 12(6): 1718.

155. *Santos TGFTD, Brito DHS, Santos NMVD, Paiva MC, Lyra MCA, Heimer MV,* et al. Viral symptoms in children and SARS-COV-2: information for pediatric dentists for the control of transmission. Braz Oral Res 2022; 36: e029.

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Surgical treatment of renal tumor with tumor thrombus in the *inferior vena cava*

Hirurško lečenje tumora bubrega sa tumorskim trombom u donjoj šupljoj veni

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Abstract

Background/Aim. An aggressive approach with radical nephrectomy and thrombectomy is the mainstay of the treatment in patients with renal tumors. The aim of this study was to present the results of such surgical procedures performed in the last 25 years at our institution. **Methods.** We made a retrospective analysis of radical nephrectomy and thrombectomy in patients with renal tumor and tumor thrombus (TT) extending into the *inferior vena cava* (IVC) operated on at our institution between January 1995 and October 2021. **Results**. There were 92 patients (72 males and 20 females) aged 60.5 on average who were operated on in the mentioned period. A predominance of right-sided tumors was present in 73.33% of patients. Patients with TT in the renal vein (levels 0 and I) were not included. TT levels II, III, and IV were present in 32 (34.8%), 52 (56.5%), and 8 (8.7%) patients, respectively. One patient had thrombosis of the right pulmonary artery. Four patients had liver metastases, and ten had lymph node involvement. The surgical approach by subcostal incision was achieved in 8 (8.69%) patients, by chevron incision in only 11 (11.95%) patients, while in 73 (79.34%) patients, we performed median sternotomy and subcostal/chevron incision. Intraoperatively, there was one complication which was pulmonary thromboembolism. Six patient required re-exploration after the surgery due to the IVC hemorrhage. The three-year survival in patients with renal tumors and TT levels II–IV in the IVC was 43%. **Conclusion**. Surgery will remain the primary cure method in patients with renal tumors and TT in the IVC. Long-term survival in these patients can be achieved by complete surgical removal (radical nephrectomy and thrombectomy).

Key words:
kidney neoplasms; neoplasm metastasis; vena cava, inferior; surgical procedures, operative; survival.

Apstrakt

Uvod/Cilj. Agresivan pristup sa radikalnom nefrektomijom i trombektomijom je osnova lečenja bolesnika sa tumorom bubrega. Cilj rada bio je da se prikažu rezultati takvih hirurških zahvata, sprovedenih tokom poslednjih 25 godina u našoj ustanovi. **Metode.** Izvršena je retrospektivna analiza radikalne nefrektomije i trombektomije kod bolesnika sa tumorom bubrega i tumorskim trombom (TT) koji se protezao u donju šuplju venu (DŠV). Prikazani bolesnici su lečeni u našoj ustanovi između januara 1995. i oktobra 2021. godine. **Rezultati**. U navedenom periodu operisana su 92 bolesnika (72 muškarca i 20 žena), prosečne starosti od 60,5 godina. Kod 73,33% bolesnika postojala je predominacija desnostranog tumora. Bolesnici sa TT u bubrežnoj veni (nivoa 0 i I) nisu bili uključeni. Nivoi TT II, III i IV bili su prisutni kod 32 (34,8%), 52 (56,5%) i 8 (8,7%) bolesnika, redom. Jedan bolesnik imao je tromb u desnoj plućnoj arteriji. Četiri bolesnika imala su metastaze u jetri, a kod deset bolesnika bili su zahvaćeni limfni čvorovi. Hirurški pristup subkostalnom incizijom bio je postignut kod 8 (8,69%) bolesnika, *chevron* rezom kod 11 (11,95%) bolesnika, dok je kod 73 (79,34%) bolesnika bila urađena sternotomija i subkostalna/*chevron* incizija. Intraoperativno, postojala je jedna komplikacija u vidu plućne

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tromboembolije. Kod šest bolesnika bilo je potrebno izvršiti reoperaciju zbog krvarenja iz operativne rane. Trogodišnje preživljavanje bolesnika sa tumorom bubrega i TT nivoa II−IV u DŠV iznosilo je 43%. **Zaključak.** Operacija će ostati primarni metod lečenja bolesnika sa tumorom bubrega i TT u DŠV. Dugotrajno preživljavanje tih bolesnika može se postići samo potpunim hirurškim uklanjanjem (radikalna nefrektomija i trombektomija).

Ključne reči:
bubreg, neoplazme; neoplazme, metastaze; v. cava inferior; hirurgija, operativne procedure; preživljavanje.

Introduction

The most common type of kidney malignancy is renal cell carcinoma (RCC), and it is twice as common in men than in women. RCC ranks sixth among all malignancies in men and seventh in women [1]. This malignancy is associated with proliferation into the *inferior vena cava* (IVC) in 4–10% of cases, and in 1% of patients, the tumor thrombus (TT) spreads to the right atrium [2–4]. Patients with RCC and TT in the IVC treated with nephrectomy alone without thrombus evacuation have a poor prognosis and die within one year from diagnosis [5]. Modern surgery imposes multidisciplinary treatment of diseases that, until recently, were the subject of only certain specialties. In addition to the urologist who treats the underlying disease and performs nephrectomy, the operative team also consists of surgeons of other specialties, primarily vascular surgeons, as well as cardio and abdominal surgeons. The operation is performed in the cardiovascular room due to the possible cardiopulmonary bypass (CPB). The anesthesiologist must be familiar with cardiovascular and thoracic surgeries and use cardiovascular arrest, extracorporeal circulation (ECC), and venous bypass [6, 7].

The aim of this study was to present the results of radical nephrectomy and thrombectomy in patients with renal tumor and TT extending into the IVC performed in the last 25 years at our institution.

Methods

From January 1995 to October 2021 at the Clinic of Urology and the Clinic for Vascular and Endovascular Surgery of the Military Medical Academy in Belgrade, Serbia, patients with RCC and TT in the IVC were treated with radical nephrectomy, including extraction of TT from the IVC. Although RCC can be present in a variety of clinical forms, from asymptomatic, accidental findings to metastatic manifestations, patients with TT most often have symptoms presented [2]. Local pain and hematuria may be the result of local tumor growth. Other symptoms include nausea, fatigue, and weight loss as a part of the paraneoplastic syndrome. Specific symptoms may appear due to the IVC occlusion, lower extremity edema, new varicocele, pulmonary thromboembolism (PTE), ascites, Budd-Chiari syndrome, *caput medusae*, cardiac dysfunction, and symptoms of metastatic disease [2, 6, 7]. Ultrasound is used for quick orientation along with the clinical picture and symptoms. With good visualization and dimensions of the tumor in the kidney, it is necessary to look for infiltration of adipose retroperitoneal tissue, lymphadenopathy, and intraabdominal metastases. Initial diagnosis includes multislice computed tomography (MSCT) and portocavography; however, magnetic resonance imaging (MRI) finding also reveals TT enlargement and degree of the IVC occlusion. Transthoracic echocardiography is used to confirm the presence of TT in the right atrium. Classification of TT levels is extremely important because it is the basis for surgery planning. Montie et al. [8] from the Mayo Clinic classify TT levels as shown in Table 1. We used that classification to estimate thrombus levels.

Surgical technique

The aim of the surgical treatment of renal tumors involving TT in the IVC is to remove all tumor tissue. With nephrectomy of the affected kidney, it is necessary to perform the IVC thrombectomy or its resection with or without reconstruction, retroperitoneal lymphadenectomy, and metastasis removal. The patient lies on their back with arms outstretched under endotracheal anesthesia. The procedure begins with a medial sternotomy and the IVC control in the supradiaphragmatic, intrapericardial part just before the IVC enters the right atrium. The IVC clamping or tourniquet in this section prevents PTE during IVC surgery and manipulation. The incision on the anterior abdominal wall is usually subcostal or bi-subcostal (chevron) but can also be approached by medial laparotomy. The decision on the unilateral subcostal incision or chevron approach is made depending on the location (side) and size of the tumor. All patients with TT levels II and III are operated on through a

Table 1

Classification of tumor thrombus (TT) levels in patients with renal tumor and TT in the *inferior vena cava* (IVC) [8]

TT level	Definition
0	Thrombus confined to the renal vein or on a pathological preparation.
I	Thrombus spreads in the IVC < 2 cm above the renal vein.
II	Thrombus spreads > 2 cm above the renal vein but below the hepatic veins.
III	Thrombus at or above the hepatic veins but below the diaphragm.
IV	Thrombus spreads above the diaphragm.

unilateral (right kidney)/bilateral-chevron (left kidney) subcostal incision and sternotomy. First, the renal artery is ligated because it results in the mobilization of the kidney with the tumor, reduces bleeding, and often retracts TT [9]. The IVC thrombectomy depends on the TT level. All patients are treated with a low dose of heparin for five to seven days postoperatively.

Tumor thrombus levels 0 and I

TT levels 0 and I enter the IVC minimally and do not obstruct it, so there is no need for sternotomy and PTE protection. These levels were excluded from this study because a vascular and/or cardiac surgeon was not involved in operation procedures in these cases. Accordingly, this analysis was focused on higher levels of malignant TT propagation.

Tumor thrombus levels II and III

TT level II requires more extensive IVC dissection to achieve thrombus themes. The upper border of the thrombus is usually determined by gentle palpation, less often with ultrasound. Unequivocally, it is necessary to put vascular loops on the subhepatic IVC, infrarenal IVC, and both renal veins to secure the patient from massive vein bleeding. This technique provides complete vascular control but does not prevent PTE if used without protection. Sometimes, it is necessary to ligate accessory hepatic veins from the caudate lobe to make a place for the subhepatic clamp. Pringle's maneuver interrupts blood flow to the liver through the portal vein and hepatic artery, thus reducing bleeding from hepatic veins after thrombus extraction and preventing liver congestion during the IVC clamping [10]. At TT levels II and III, an L-shaped IVC incision is made from the renal vein confluence over the IVC to the proximal [9]. TT is gently separated with fingers or a vascular instrument with a dissector (probe), and the renal vein ostium is cut off and can often be removed as a single specimen. Due to the fragile consistency of TT, it often disintegrates and removes several parts. The IVC lumen is washed with saline, and any remaining thrombus is inspected and subsequently extirpated. Part of the IVC wall around the ostium is biopsied and sent for histological verification. TT is often extirpated completely because a smaller thrombus does not adhere to the IVC wall; however, a larger thrombus that completely obstructs the IVC and reaches the hepatic veins adheres, leaving a thin layer fixed to the IVC wall. In that case, resection of the IVC with reconstruction should be considered. After thrombectomy, the tourniquet on the hepatoduodenal ligament (Pringle) is loosened to flush out any remaining thrombus and air, and the vascular clamp is placed infrahepatically. The hole on the IVC is sewn with polypropylene suture (Prolen® 4–0). The last suture is left untouched so that after releasing the remaining clamps, the bloodstream can expel any blood clots and air, after which it is tightened [11, 12]. At the moment of releasing the clamp, an anesthesiologist helps by increasing the pressure in the pulmonary bloodstream to ensure a constant venous inflow and ventilation of the IVC. If a blood thrombus is present along with the tumor, the patient is prescribed anticoagulant therapy in order to prevent venous thrombosis and the focus of thromboembolism.

Tumor thrombus level IV

TT level IV is the most complicated part of the surgical treatment of TT in the IVC. The safest and most common way to extirpate a tumor from the right atrium would be through a sternotomy, with CPB and arrest. After establishing the ECC, a longitudinal incision about 3 cm long is made on the front of the right atrium. If the thrombus is larger, then extraction is performed, and if it is smaller and not fixed, it is possible to manually push it back into the IVC and place a vascular clamp or tourniquet above it. In the second act of the operation, the thrombus is removed, as in the case of TT level III, through an abdominal incision. This maneuver is useful in compact tumors that have not adhered to the atrial wall and when the incision is performed on a beating heart without CPB. After extraction or distal repositioning, the incision is closed with Prolen® 4–0 suture. The clamp or tourniquet on the IVC near the atrium wall must stand until the end of the operation so that we do not artificially provoke PTE.

Anesthesiological technique

All patients underwent surgery under balanced general endotracheal anesthesia. After standard premedication, intravenous anesthesia (benzodiazepines, opioids, intravenous anesthetic, and muscle relaxant) is administered. An inhalation anesthetic (sevoflurane) is used as a standard to maintain anesthesia with the additional use of opioids and muscle relaxants. In most patients, unless there are contraindications or technical limitations, an epidural catheter is used for intraoperative and postoperative analgesia. For patients with levels II, III, and IV thrombus propagations into the IVC, epidural morphine in a dose of 2–4 mg is used with local anesthetics. In addition to standard monitoring, invasive monitoring is applied (arterial line for invasive continuous measurement of arterial pressure, central venous catheter for monitoring central venous pressure). All patients undergo a nasogastric tube at the introduction. Patients are heated during surgery using external heaters and heated intravenous fluid infusions. Vasopressors (noradrenaline or phenylephrine) are used to maintain perfusion pressure during the critical phases of the operation (clamping or placing the ligature on the IVC at different heights). Activating clotting time monitoring is used intraoperatively to monitor anticoagulation (heparin) in patients with TT level IV. At other levels, intraoperative use of heparin is not used. According to the indication, viscoelastic tests (Rotational Thromboelastometry – ROTEM) with targeted correction of hemostasis disorders are used in the patients. All patients are scheduled for extubation within 6–8 hrs after completing the intervention.

Results

In our series, we operated on 92 patients (72 men and 20 women) with renal tumors that invaded the IVC, with an average age of 60.5 years (32 to 81 years). There was a right-sided dominance of the tumor in 73.33% of patients. TT levels II, III, and IV were present in 32 (34.8%), 52 (56.5%), and 8 (8.7%) patients, respectively. Localization of renal tumor and TT level, confirmed by MSCT, is shown in Figure 1.

The operative approach involved a subcostal incision in 8 (8.69%) patients and a chevron (bilateral subcostal incision) in 11 (11.95%) patients. In the remaining 73 (79.34%) patients, the surgical approach was subcostal (right kidney) /chevron (left kidney) with sternotomy due to PTE protection (Figure 2).

In one case, we performed the IVC restraint through an anterior right thoracotomy because the patient had previously had a sternotomy with aortocoronary bypass surgery. All patients underwent radical nephrectomy with the extraction of the TT from the IVC (Figure 3).

In 92.3% of patients, RCC was histopathologically confirmed, while lymphoma, Ewing sarcoma, and transitional cell carcinoma were present in 2.2% of patients and adult Wilms tumor in 1.1% of patients. Preoperatively, 87% of patients had significant comorbidity, of which 52.1% had cardiac disease, while 19.6% had diabetes mellitus.

CPB was used four times in patients with TT level IV, where TT filled the entire atrium, and all passed without complications (Figure 4).

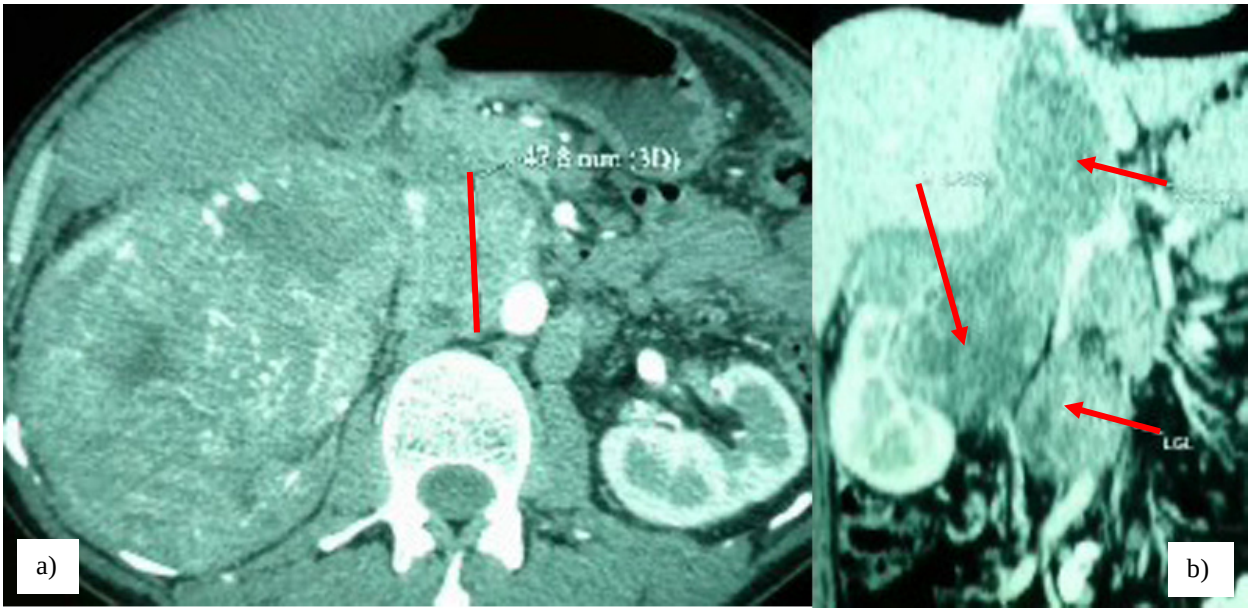

Fig. 1 – Multi-slice computed tomography cavography. a) The *inferior vena cava* (IVC), 47 mm in diameter, filled completely with tumor thrombus (TT); b) Diaphragm level (TT level III): tumor (upper left arrow), IVC with TT (upper right arrow), lymph node (lower right arrow).

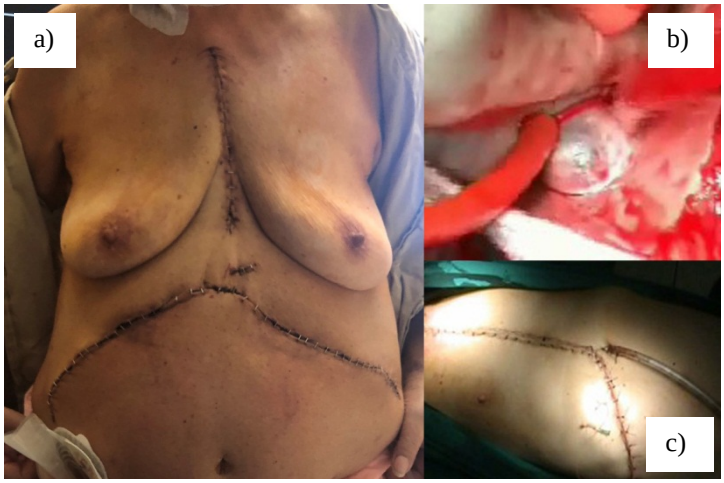

Fig. 2 – a) Sternotomy and chevron for left-sided tumor and tumor thrombus (TT) level III; b) Loop on the *inferior vena cava* beneath the right atrium; c) Sternotomy and right subcostal incision for right-sided tumor and TT level III.

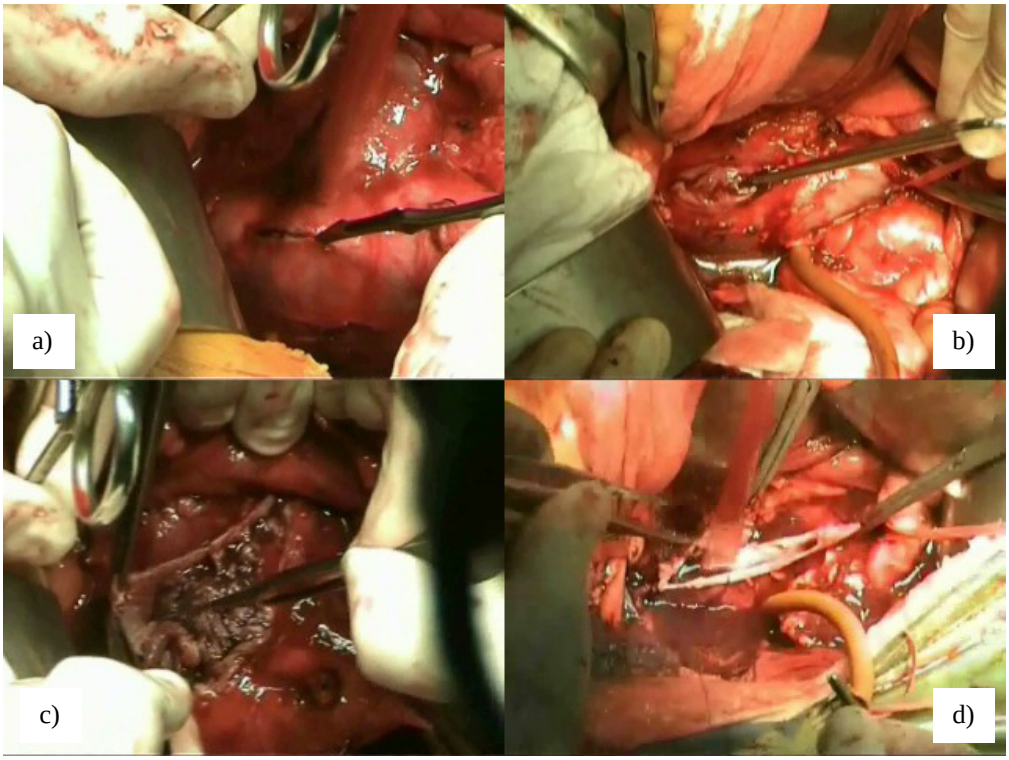

Fig. 3 – a) The *inferior vena cava* (IVC) anterior incision; b) Completed IVC incision; c) Thrombus extirpation; d) Sewing the hole on the IVC.

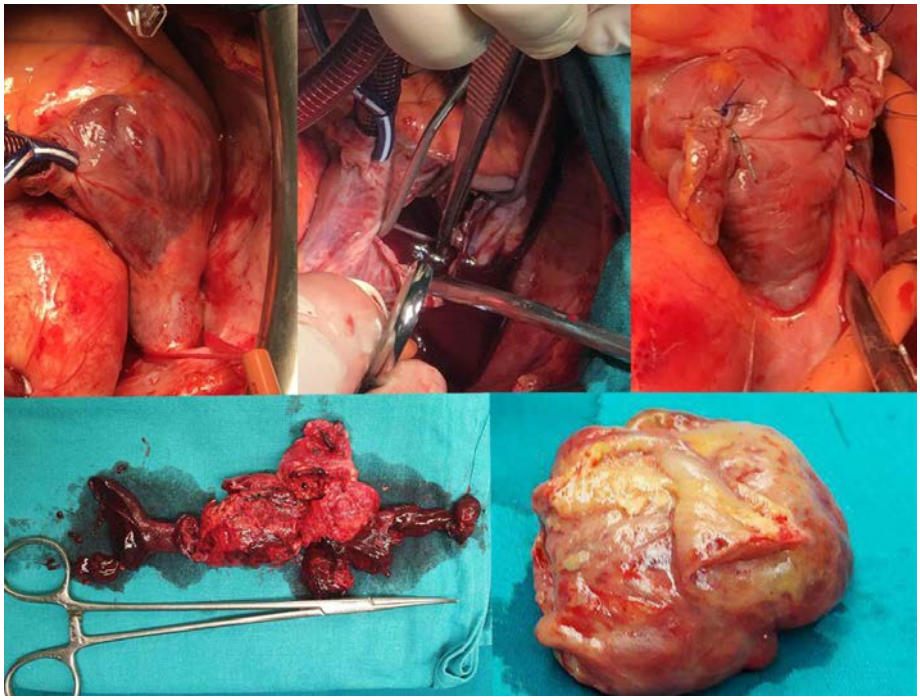

Fig. 4 − Extirpation of tumor thrombus from the right atrium (upper parts of the display), extirpated thrombus in its entirety (lower parts of the display). Extracorporeal circulation was applied.

With the "beating heart" technique, we operated on three patients without complications; one case was operated on an intermittent cardiac arrest.

The average duration of the operation was 180 min. Intraoperative and early postoperative blood loss initially ranged from 500 to 15,000 mL; the average dose of blood replacement was four units (1−26 units). In the last five years, due to the developed technique, the bleeding during operation has been minimal and controlled, and it does not exceed 1,000 mL of blood with four units of compensation. No balloon occlusion with transesophageal echocardiography was used. There was no perioperative

mortality. The average number of hospital days was 14. The follow-up period covered, in the best case, 36 months from the operation due to the breakdown of a patient. In nine (9.78%) cases, an invasion of the excised IVC wall was noted (Figure 5).

Ten patients had tumor-altered lymph nodes in the interaortocaval and retrocaval positions. Four patients had solitary metastases in the liver, and one patient had, in addition to the TT level III, an echinococcal cyst that was extirpated in the same act. Six (6.52%) patients required re-exploration after surgery due to bleeding. All bleeding was diffuse in nature when hemostasis was established by the replacement of blood elements and plasma. Six splenectomies were reported. There were four cases of subsequent blood thrombosis of the IVC without clinical significance. We had one iatrogenic intraoperative PTE when we did not use protection during sternotomy. By prompt reaction, we extracted a thrombus from the pulmonary artery, and the patient (34 years old) survived the operation without consequences. Late complications from 30 days to one year after the surgery included chronic kidney insufficiency in about a third of operated patients and recurrences of the underlying disease. Eight recurrences of TT in the IVC were reported (two TT level IV, five TT level III, and one TT at the incision site). TT levels III and IV recurrences due to advanced disease were not reoperated. A patient who developed a recurrence at the IVC incision site after three months was reoperated. The reoperation revealed the entry of TT through the lumbar vein, which was free in the primary operation. The patient was alive 13 years after reoperation without signs of recurrence [13]. Estimation of survival was difficult because about 25% of the patients did not report for check-ups at our institution due to the distance from our Center and the COVID-19 pandemic in the last two years. According to the available result findings, due to renal tumor with TT in the renal vein and/or IVC, the total three-year survival rate in our series of all patients operated on was 81%, which is not significantly different from the survival of renal tumor patients without TT invading the IVC. For the isolated group of patients with TT levels II–IV, for which data were available, the three-year survival rate was 43%. In 10% of these patients, the fatal outcome was due to diseases unrelated to the kidney tumor.

Discussion

In 4–10% of cases, untreated kidney tumors get complicated by the formation of TT in the renal vein that spreads to the right atrium in 1% of patients and PTE with a poor outcome. The median survival time of the non-operated patients is five months, and the one-year survival rate is 29% [14]. Significantly better survival is achieved by evacuating the TT from the IVC with nephrectomy, especially in the absence of distant metastases and regional

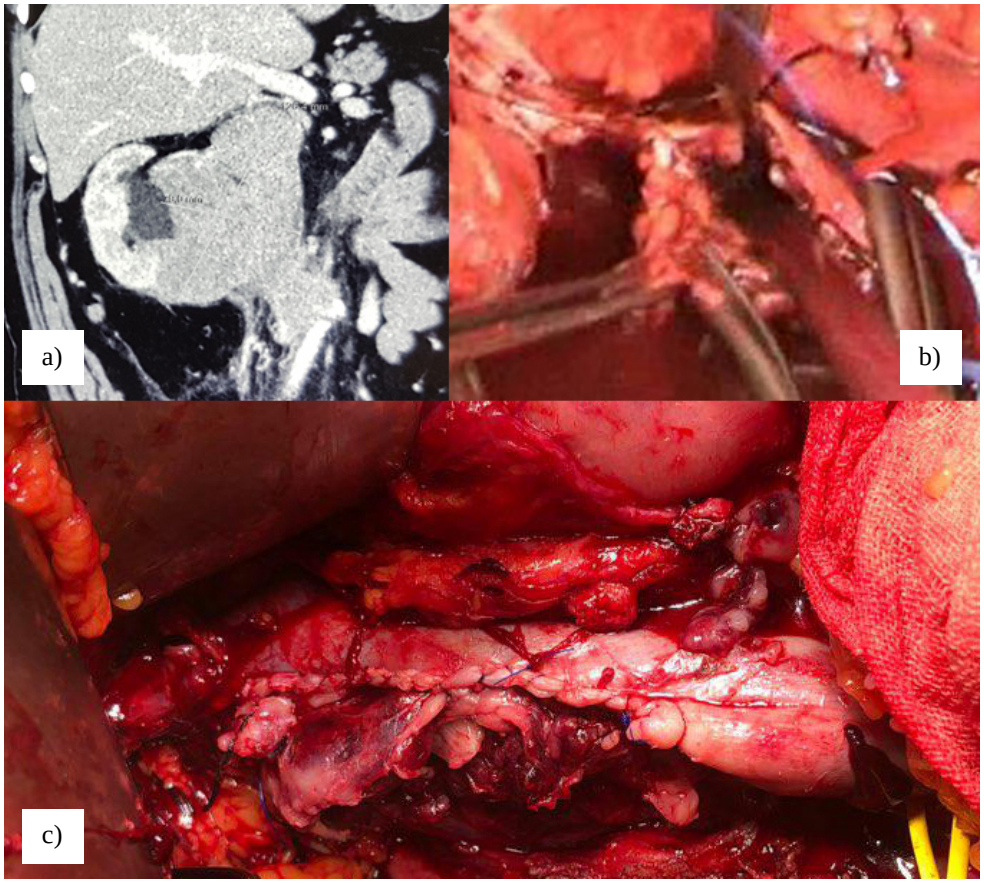

Fig. 5 – a) Multi-slice computed tomography of the right kidney and tumor thrombus (TT); b) Extraction of TT and biopsy of *inferior vena cava* (IVC); c) Sewed IVC with clear signs of tumor infiltration.

lymph node involvement. With this treatment of TT levels II–IV, three- and five-year survival rates are up to 39–43% and 58–60%, respectively [9, 15–17]. By combining the knowledge of several surgical specialties, far more significant and better results can be achieved in the group of patients who were, until recently, incurable [18]. For these reasons, radical nephrectomy with the IVC thrombectomy has become the gold standard in treating these patients, with a two-to-five years' survival rate of 32−64% [10, 18]. This operation presents a challenge for the surgeon due to potential massive bleeding and tumor thromboembolism [19]. Advanced intraoperative and postoperative anesthesia support allows far more aggressive surgical approaches and procedures [19, 20]. With the advancement of immunotherapy [21, 22], control of distant metastases can be achieved in patients with renal tumors spreading into the IVC so that their survival is prolonged if more aggressive surgical therapy is performed [23]. Retrohepatic and supradiaphragmatic localization of TT is a real challenge for the surgeon. The proximity and possible involvement of the hepatic veins, the proximity of the right atrium, and the poor exposure of the retrohepatic part of the IVC are the facts that make this procedure extremely difficult to perform. One technique cannot be applied to all patients, and a surgical plan is made for each patient individually. The optimal procedure is planned based on preoperative diagnostics, especially according to the level of occlusion and the added blood clot distally. Typically, the TT level IV involves CPB and circulatory arrest so that the tumor can be easily extirpated from the atrium [7]. However, if surgery is continued under the CPB, increased bleeding may occur due to nephrectomy because the patient is completely heparinized. That prolongs the time of surgery and anesthesia and increases the risk of postoperative coagulopathy. In our patients, after extirpation of TT from the atrium, we discontinued CPB and reversed the effect of heparin with an adequate dose of protamine. Renal artery embolization can be performed preoperatively to reduce blood loss and achieve the reduction of tumor and TT partially, transferring it to a lower stage [24]. We used this procedure four times at the beginning of the series, but we significantly reduced the bleeding by improving the surgical technique. Prevention of PTE can be achieved by placing an endoluminal balloon catheter [25]. However, there is a great risk of the IVC wall bursting, and passing a catheter through TT can lead to embolization. Furthermore, some authors suggested a transabdominal approach to avoid CPB and sternotomy by liver mobilization and transdiaphragmal path to the pericard and the IVC control [26–28]. In our series, prevention of distal embolization was achieved by placing a tourniquet on the IVC just below the right atrium through a sternotomy. Isolated sternotomy, as protection against PTE, is proven to be an extremely safe and effective procedure. Suspected invasion of the IVC wall probably requires partial or total resection with the IVC vascular reconstruction to maximize cancer control [29, 30]. In our patients, we did not resect the IVC, so there was no need for placing a graft. Alternatively, fully occluded IVC can be safely ligated, or IVC resection (cavectomy) can be performed without significant morbidity [30], but we did not use that method in our series. Postoperatively, in patients with IVC TT, low-dose heparin therapy was prescribed for a period of five to seven days. About 15% of patients have early complications up to 30 days after the operation. Perioperative mortality involves about 2–3% of patients [1], and up to 7.5% occur due to hemorrhagic complications [3]. The need for transfusion during and immediately after the surgery increases with TT levels. Early complications are associated with TT levels. As many as 46.9% of patients with TT level IV have early complications compared to level 0, where 12.4% of patients experience early complications. Splenectomy and IVC thrombosis are not rare [25]. Although some authors show that the ten-year survival rate is significantly lower if the thrombus is above the diaphragm [3], there is no significant difference in the patient's outcome depending on the TT level [7]. Nonmetastatic renal tumor with spread into the IVC is potentially curable if complete tumor removal is performed [31].

Conclusion

The treatment outcome has been improved, thus reducing morbidity and mortality. The progress in reducing morbidity and mortality and in improving the treatment outcome of patients with renal tumors and TT invading the IVC is provided by an aggressive surgical procedure. This procedure can also be used if there are resectable distant metastases; it can also be performed as a palliative procedure. Preoperative determination of TT levels is crucial in planning surgery for kidney tumors with thrombus in the IVC. Sternotomy used in our patients did not increase intraoperative mortality and morbidity compared to the other studies in which only the transabdominal approach was used. PTE with TT is the most severe perioperative complication, with a mortality rate of up to 75%. The use of IVC control through sternotomy eliminates the risk of intraoperative PTE.

Surgery will remain the primary method of treatment in cases where renal tumors involve the IVC with TT. Long-term survival in these patients can only be achieved by complete surgical removal (radical nephrectomy and thrombectomy). Until now, these patients have been considered inoperable with a fatal outcome within one year, so this procedure significantly improves the results of their treatment outcome.

R E F E R E N C E S

1. *Siegel RL, Miller KD, Jemal A*. Cancer statistics, 2019. CA Cancer J Clin 2019; 69(1): 7–34.
2. *Marshall VF, Middleton RG, Holswade GR, Goldsmith EI*. Surgery for renal cell carcinoma in the vena cava. J Urol 1970; 103(4): 414–20.
3. *Kim HL, Zisman A, Han KR, Figlin RA, Belldegrun AS*. Prognostic significance of venous thrombus in renal cell carcinoma. Are renal vein and inferior vena cava involvement different? J Urol 2004; 171(2 Pt 1): 588–91.
4. *Chiappini B, Savini C, Marinelli G, Suarez SM, Di Eusanio M, Fiorani V,* et al. Cavoatrial tumor thrombus: single-stage surgical approach with profound hypothermia and circulatory arrest, including a review of the literature. J Thorac Cardiovasc Surg 2002; 124(4): 684–8.
5. *Sosa RE, Muecke EC, Vaughan ED Jr, McCarron JP Jr*. Renal carcinoma extending into the inferior vena cava: the prognostic significance of the level of vena caval involvement. J Urol 1984; 132(6): 1097–100.
6. *Masatoshi J, Takehisa I, Yoshinori I, Norihide S, Kazunori K, Nobuhiko H*, et al. Surgical strategy for treating renal cell carcinoma with thrombus extending into the inferior vena cava. J Vasc Surg 2004; 39(4): 829–35.
7. *Abel E, Thompson R, Margulis V, Heckman J, Merril M, Darwish O*, et al. Perioperative outcomes following surgical resection of renal cell carcinoma with inferior vena cava thrombus extending above the hepatic veins: a contemporary multicenter experience. Eur Urol 2014; 66(3): 584–92.
8. *Montie JE, el Ammar R, Pontes JE, Medendorp SV, Novick AC, Streem SB*, et al. Renal cell carcinoma with inferior vena cava tumor thrombi. Surg Gynecol Obstet 1991;173(2): 107–15.
9. *Blute M, Leibovich B, Lohse C, Cheville J, Zincke H*. The Mayo Clinic experience with surgical management, complications and outcome for patients with renal cell carcinoma and venous tumour thrombus. BJU Int 2004; 94(1): 33–41.
10. *Pringle JH*. V. Notes on the Arrest of Hepatic Hemorrhage Due to Trauma. Ann Surg 1908; 48(4): 541–9.
11. *Dellaportas D, Arkadopoulos N, Tzanoglou I, Bairamidis E, Gemenetzis G, Xanthakos P,* et al. Technical Intraoperative Manoeuvres for the Management of Inferior Vena Cava Thrombus in Renal Cell Carcinoma. Front Surg 2017; 4: 48.
12. *Rehman ZU, Ather MH, Aziz W*. Surgical Interventions for Renal Cell Carcinoma with Thrombus Extending into the Inferior Vena Cava: A Multidisciplinary Approach. Ann Vasc Dis 2019; 12(1): 55–9.
13. *Tomić A, Milović N*. Recidive of renal cell carcinoma tumor thrombus in inferior vena cava via lumbar vein. Int Surg 2010; 95(4): 366–70.
14. *Reese A, Whitson J, Meng M*. Natural history of untreated renal cell carcinoma with venous tumor thrombus. Urol Oncol 2013; 31(7): 1305–9.
15. *Haddad A, Wood C, Abel E, Krabbe L, Darwish O, Thompson R*, et al. Oncologic outcomes following surgical resection of renal cell carcinoma with inferior vena caval thrombus extending above the hepatic veins: a contemporary multicenter cohort. J Urol 2014; 192(4): 1050–6.
16. *Ciancio G, Manoharan M, Katkoori D, De Los Santos R, Soloway MS*. Long-term survival in patients undergoing radical nephrectomy and inferior vena cava thrombectomy: single-center experience. Eur Urol 2010; 57(4): 667–72.
17. *Chen Z, Yang F, Ge L, Qiu M, Liu Z, Liu C*, et al. Outcomes of renal cell carcinoma with associated venous tumor thrombus: experience from a large cohort and short time span in a single center. BMC Cancer 2021; 21(1): 766.
18. *Rigaud J, Hetet JF, Braud G, Battisti S, Le Normand L, Glemain P*, et al. Surgical Care, Morbidity, Mortality and Follow-up after Nephrectomy for Renal Cancer with Extension of Tumor Thrombus into the Inferior Vena Cava: Retrospective Study Since 1990s. Euro Urol 2006; 50(2): 302–10.
19. *Novick AC, Kaye MC, Cosgrove DM, Angermeier K, Pontes JE, Montie JE,* et al. Experience with cardiopulmonary bypass and deep hypothermic circulatory arrest in the management of retroperitoneal tumors with large vena caval thrombi. Ann Surg 1990; 212(4): 472–6; discussion 476–7.
20. *Kuczyk MA, Münch T, Machtens S, Grünewald V, Jonas U*. The impact of extracorporal circulation on therapy-related mortality and long-term survival of patients with renal cell cancer and intracaval neoplastic extension. World J Urol 2002; 20(4): 227–31.
21. *Bhat A, Nahar B, Venkatramani V, Banerjee I, Kryvenko ON, Parekh DJ*. Metastatic Renal Cell Carcinoma with Level IV Thrombus: Contemporary Management with Complete Response to Neoadjuvant Targeted Therapy. Case Rep Urol 2019; 2019: 7102504.
22. *Oltean AM, Matuz R, Sitar-Taut A, Mihailov A, Rednic N, Tantau A*, et al. Renal Cell Carcinoma With Extensive Tumor Thrombus Into the Inferior Vena Cava and Right Atrium in a 70-Year-Old Man. Am J Mens Health 2019; 13(3): 1557988319846404.
23. *Naitoh J, Kaplan A, Dorey F, Figlin R, Belldegrun A*. Metastatic renal cell carcinoma with concurrent inferior vena caval invasion: long-term survival after combination therapy with radical nephrectomy, vena caval thrombectomy and postoperative immunotherapy. J Urol 1999; 162(1): 46–50.
24. *Schwartz MJ, Smith EB, Trost DW, Vaughan ED Jr*. Renal artery embolization: clinical indications and experience from over 100 cases. BJU Int 2007; 99(4): 881–6.
25. *Zini L, Haulon S, Leroy X, Christophe D, Koussa M, Biserte J,* et al. Endoluminal occlusion of the inferior vena cava in renal cell carcinoma with retro- or suprahepatic caval thrombus. BJU Int 2006; 97(6): 1216–20.
26. *Ciancio G, Soloway MS*. Renal cell carcinoma with tumor thrombus extending above the diaphragm: avoiding cardiopulmonary bypass. Urology 2005; 66(2): 266–70.
27. *Taweemonkongsap T, Nualyong C, Leewansangtong S, Amornvesukit T, Sirivatanauksorn Y, Tantiwong A,* et al. Surgical treatment of renal cell carcinoma with inferior vena cava thrombus: using liver mobilization technique to avoid cardiopulmonary bypass. Asian J Surg 2008; 31(2): 75–82.
28. *Ciancio G, Livingstone AS, Soloway M*. Surgical Management of Renal Cell Carcinoma with Tumor Thrombus in the Renal and Inferior Vena Cava: The University of Miami Experience in Using Liver Transplantation Techniques. Eur Urol 2007; 51(4): 988–95; discussion 994–5.
29. *Blute ML, Boorjian SA, Leibovich BC, Lohse CM, Frank I, Karnes RJ*. Results of inferior vena caval interruption by greenfield filter, ligation or resection during radical nephrectomy and tumor thrombectomy. J Urol 2007; 178(2): 440–5; discussion 444.
30. *Ciancio G, Soloway M*. Resection of the abdominal inferior vena cava for complicated renal cell carcinoma with tumour thrombus. BJU Int 2005; 96(6): 815–8.
31. *Bissada NK, Yakout HH, Babanouri A, Elsalamony T, Fahmy W, Gunham M*, et al. Long-term experience with management of renal cell carcinoma involving the inferior vena cava. Urology 2003; 61(1): 89–92.

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Risk factors for cerebral palsy

Faktori rizika od nastanka cerebralne paralize

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Abstract

Background/Aim. Cerebral palsy (CP) etiology is multifactorial and heterogeneous, manifesting as damage to the developing brain. The associated risk factors can arise in the prenatal, perinatal, or postnatal period. The aim of this study was to determine the risk factors for CP and examine the associations between CP type, gestational age, and perinatal risk factors. **Methods.** The study sample comprised 206 children with CP. Pertinent data were collected from medical records and included participants' gestational age at birth, medical history, and CP clinical characteristics. Risk factors were divided according to the timing of brain injury into prenatal, perinatal, and neonatal. **Results.** Hormonally maintained pregnancy (55.3%), twin pregnancy (28.9%), vaginal bleeding after the 20th week of gestation (21.1%), threatened abortion in the first half of pregnancy (13.2%), and maternal infection (10.5%) were identified as the main prenatal risk factors for CP. Prematurity (54.5%) was the leading perinatal risk factor, followed by low birthweight (50.8%), Apgar score < 7 (41.7%), assisted delivery (41.4%), and breech presentation (13.5%). Respiratory distress syndrome (16%), need for treatment in the Neonatal Intensive Care Unit (22.3%), assisted ventilation (18.4%), hypoxic-ischemic encephalopathy (11.2%), and neonatal convulsions (5.8%) were identified as the leading neonatal risk factors for CP. A statistically significant difference was found in the total number of perinatal risk factors in relation to gestational age ($p < 0.001$) and CP type ($p = 0.006$). Perinatal risk factors were most prevalent in preterm infants and children affected by the CP of spastic bilateral type. A statistically significant difference was noted in the distribution of CP types depending on the gestational age ($p < 0.001$). In particular, spastic bilateral CP type was most prevalent in the group of preterm-born children. **Conclusion.** CP is characterized by heterogeneous risk factors and is a result of interaction among multiple risk factors.

Key words:
cerebral palsy; gestational age; infant, premature; pregnancy complications; pregnancy, twin; risk factors.

Apstrakt

Uvod/Cilj. Etiologija cerebralne paralize (CP) je multifaktorijalna i heterogena i karakteriše je oštećenje mozga u razvoju. Faktori rizika mogu se javiti u prenatalnom, perinatalnom i postanatalnom periodu. Cilj rada bio je da se utvrde faktori rizika od nastanka CP i istraži odnos između oblika CP, gestacijske starosti i perinatalnih faktora rizika. **Metode.** Istraživanjem je obuhvaćeno ukupno 206 dece obolelih od CP. Iz medicinske dokumentacije obolele dece dobijeni su podaci o njihovoj gestacijskoj starosti, kliničkim i ostalim karakteristikama. Faktori rizika su podeljeni prema trenutku nastanka oštećenja mozga na prenatalne, perinatalne i neonatalne. **Rezultati**. Najčešći prenatalni faktori rizika za nastanak CP bili su hormonski održavana trudnoća (55,3%), blizanačka trudnoća (28,9%), vaginalno krvarenje nakon 20. nedelje gestacije (21,1%), preteći abortus u prvoj polovini trudnoće (13,2%) i infekcija majke (10,5%). Među perinatalnim faktorima rizika najčešće su bili zastupljeni prevremeno rođenje (54,5%), praćeno niskom telesnom masom na rođenju (50,8%), Apgar skor < 7 (41,7%), asistirani porođaj (41,4%) i karlična prezentacija novorođenčeta (13,5%). Vodeći neonatalni faktori rizika za nastanak CP bili su respiratorni distres sindrom (16%), potreba za lečenjem na Odeljenju intenzivne nege i terapije (22,3%), asistirana ventilacija (18,4%), hipoksično-ishemijska encefalopatija (11,2%) i neonatalne konvulzije (5,8%). Utvrđena je statistički značajna razlika u ukupnom broju perinatalnih faktora rizika u odnosu na gestacijsku starost ($p < 0,001$) i tip CP ($p = 0,006$). Perinatalni faktori rizika bili su učestaliji kod prevremeno rođene dece i dece sa bilateralnom spastičnom formom CP. Utvrđena je statistički značajna razlika u distribuciji tipova CP u odnosu na gestacijsku starost ($p < 0,001$). U grupi

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prevremeno rođene dece najzastupljeniji je bio spastični bilateralni tip CP. **Zaključak.** Etiologija CP je heterogena i ova bolest nastaje kao rezultat interakcije mnogobrojnih faktora rizika.

Ključne reči:
cerebrana paraliza; gestacijska starost; nedonošče; trudnoća, komplikacije; trudnoća, blizanačka; faktori rizika.

Introduction

Cerebral palsy (CP) is the most severe and the most common cause of disability in childhood, and because of that, it imposes the greatest burden on the healthcare system [1]. The extent of its severity is evident from the fact that 40% of children affected by CP are unable to walk independently [2], one-third have epilepsy [3], and about half have cognitive impairments [2, 4]. As a result, the lifetime cost of CP per patient is estimated at about one million USD. Given that prenatal and perinatal complications increase the risk of CP, their prevention and recognition is a public health priority [1]. The CP prevalence is estimated at 1.5–4 cases per 1,000 live births [1, 5–8].

Although CP is a result of nonprogressive brain damage which occurs before birth, during childbirth, or in the first two years of a child's life [9], the initial brain damage most commonly arises in the early fetal period [10] as a result of a congenital brain anomaly, infection, trauma, or an acute hypoxic-ischemic stroke [9]. CP etiology is multifactorial and heterogeneous, and although it cannot be established in many cases, it manifests as damage to the developing brain [5, 10]. The associated risk factors can arise in the prenatal, perinatal, or postnatal period and include multiple pregnancies, intrauterine infections, intrauterine stasis, preterm birth, cesarean section, perinatal stroke and infection, asphyxia, placental malformations, and congenital malformations [5, 8–10]. Thus, while accurately determining and clearly categorizing CP causes is not feasible, every effort must be made to identify and evaluate possible contributing factors or causal pathways [5].

The aim of this study was to determine the risk factors for CP and investigate the association among CP type, gestational age, and perinatal risk factors.

Methods

This work is part of a larger classical clinical-epidemiological qualitative study conducted at the Clinic for Child Habilitation and Rehabilitation, the Institute for Child and Youth Health Care of Vojvodina in Novi Sad, Serbia. The main study (involving 206 children) and all its components were approved by the Ethics Committee of the Institute (No. 400/1 from March 05, 2009). The data collection methods used are already described in our previous publication [11] and focused on participants' gestational age at birth, medical history, and CP clinical characteristics. For each child, Surveillance of Cerebral Palsy in Europe (SCPE) [12] and European commission guidelines (https://eu-rd-platform.jrc.ec.europa.eu/scpe/data-collection/cp-subtypes_en) [13] were used to determine clinical CP type. The risk factors identified from patients' medical records were categorized under the prenatal, perinatal, or neonatal category, depending on the timing of the brain injury [14].

Once the data was entered into our database as previously described [11], descriptive and inferential statistics were calculated, and findings were reported as percentages and frequencies. Observed frequencies in the attributive characteristics were compared to the expected values via the χ^2 test, whereas Spearman's ρ was calculated to assess pairwise correlations between characteristics. SPSS Statistics 17.0 commercial software was used in all statistical analyses.

Results

Of the 38 children with CP for whom data on some of the aforementioned prenatal risk factors were available, in 55.3% of these cases, mothers had hormonally maintained pregnancies, in 21.1%, the mother had vaginal bleeding after the 20th gestational week, in 13.2%, the mothers had threatened abortion in the first half of pregnancy, and in 10% of the cases, the mother had an infection during pregnancy. In a smaller percentage (5.3%) of children, the mother developed anemia in the second half of pregnancy, and in 2.6% of cases, the mother had hypertension in this period (Table 1).

As shown in Table 2, premature birth (54.5%) or low birthweight (50.8%) – both of which are perinatal risk factors – were also registered in more than half of the children

Table 1

Distribution of prenatal risk factors (PRF) in children with cerebral palsy

PRF	All examined patients n = 206	Patients with any PRF n = 38
Anemia (second half of pregnancy), n = 2	1.0	5.3
Threatened abortion (first half of pregnancy), n = 5	2.4	13.2
Bleeding (vaginal) (after 20th week of gestation), n = 8	3.9	21.1
Hypertension (second half of pregnancy), n = 1	0.5	2.6
Hormonally maintained pregnancy, n = 21	10.2	55.3
Maternal infection, n = 4	1.9	10.5
Twin pregnancy, n = 12	5.8	28.9

All values are expressed as percentages.

with CP. Moreover, 41.4% and 41.7% of these children had assisted delivery and a low Apgar score (AS), while in 13.5% of cases, the fetus had a breech presentation at delivery.

Out of 145 children with CP for whom gestational age was recorded, 54.4% were born prematurely, out of which 30.3% were born after 32−36 weeks of gestation, 17.2% were delivered after 28−31 weeks of gestation, and 6.9% were born before the 28th gestational week. As shown in Table 3, reproduced from our earlier study [11], the difference in distribution is statistically highly significant (χ^2 = 48.572, $p < 0.001$).

A statistically significant difference was present in the total number of perinatal factors concerning gestational age (χ^2 = 83.459, $p < 0.001$). While 48.5% of all infants carried to term had no perinatal risk factors, at least one factor was noted for those born prematurely. Moreover, the percentage of children with at least one perinatal risk factor decreased significantly with gestational age, from 100% for children born before 28 weeks to 84% for those born after 28−31 weeks of gestation, to 84.1% for children born after 32−36 weeks of gestation, and finally to only 13.6% for children carried to term (Table 4).

As indicated in Table 5, a statistically significant difference was present in the total number of perinatal factors across clinical types of CP (χ^2 = 21.354, p = 0.006). Specifically, 92% of children with spastic diplegia and 81.8% of those with quadriplegia had at least one perinatal risk factor, whereby one perinatal factor was noted in 24% and 18.2% of those cases, respectively, while the remaining 68% and 63.6% had two or more perinatal factors. The presence of perinatal risk factors was less common in children with other clinical types of CP.

Data reported in Table 6 further demonstrate the presence of a statistically significant difference in the distribution of clinical types of CP in relation to gestational age (χ^2 = 33.448, $p < 0.001$). While 71.8% of children with spastic unilateral CP were born at term, this percentage slightly declined for those with the dyskinetic (69.2) and ataxic type (66.7%). In contrast, children with a spastic bilateral type of CP were more likely to have been born prematurely, which is the case for 85.4% and 51.6% of children with diplegia and quadriplegia, respectively.

Table 2

Distribution of prenatal risk factors (PRF) for cerebral palsy

PRF	All examined patients n = 206		Patients with any PRF n = 152
	%	valid %	%
Placental disorders, n = 6	2.9	-	3.9
Assisted delivery, n = 46	22.3	41.4	30.3
Breech presentation, n = 7	3.4	13.5	4.6
Prematurity, n = 79	38.3	54.5	52.0
Apgar score < 7, n = 40	19.4	41.7	26.3
Low birthweight, n = 62	30.1	50.8	40.8
Resuscitation, n = 25	12.1	-	16.4

Table 3

Distribution of children with cerebral palsy by gestational age

Gestational age (in weeks)	Total number of patients n=206	Patients with complete data n=145
< 28, n = 10	4.9	6.9
28−31, n = 25	12.1	17.2
32−36, n = 44	21.4	30.3
> 36, n = 66	32.0	45.5
Total, n = 145	70.4	100.0
No data, n = 61	29.6	

All values are expressed as percentages.
Note: Table adopted from our previous work [11].

Table 4

The total number of perinatal risk factors (PRF) concerning gestational age

Gestational age (in weeks)	Number of PRF			Total
	0	1	≥ 2	
< 28			10 (100)	10 (100)
28−31		4 (16)	21 (84)	25 (100)
32−36		7 (15.9)	37 (84.1)	44 (100)
>36	32 (48.5)	25 (37.9)	9 (13.6)	66 (100)
Total	32 (22.1)	36 (24.8)	77 (53.1)	145 (100)

All values are expressed as numbers (percentages).

Table 5

The total number of perinatal risk factors (PRF) concerning cerebral palsy (CP) type

Clinical type of CP	Number of PRF			Total
	0	1	≥ 2	
Spastic unilateral	15 (37.5)	14 (35.0)	11 (27.5)	40 (100)
Spastic bilateral diplegia	4 (8)	12 (24)	34 (68)	50 (100)
Spastic bilateral quadriplegia	6 (18.2)	6 (18.2)	21 (63.6)	33 (100)
Dyskinetic	3 (23.1)	5 (38.5)	5 (38.5)	13 (100)
Ataxic	2 (33.3)	2 (33.3)	2 (33.3)	6 (100)
Total	30 (21.1)	39 (27.5)	73 (51.4)	142 (100)

All values are expressed as numbers (percentages).

Table 6

Distribution of cerebral palsy (CP) types by gestational age

Clinical type of CP	Preterm birth	Term birth	Total
Spastic unilateral	11 (28.2)	28 (71.8)	39 (100.0)
Spastic bilateral diplegia	41 (85.4)	7 (14.6)	48 (100.0)
Spastic bilateral quadriplegia	16 (51.6)	15 (48.4)	31 (100.0)
Dyskinetic	4 (30.8)	9 (69.2)	13 (100.0)
Ataxic	2 (33.3)	4 (66.7)	6 (100.0)
Total	74 (54.0)	63 (46.0)	137 (100.0)

All values are expressed as numbers (percentages).

Table 7

Distribution of neonatal risk factors

Neonatal risk factors	Children with CP
Respiratory distress syndrome	33 (16.0)
Hypoxic-ischemic encephalopathy	23 (11.2)
Assisted ventilation	38 (18.4)
Need for treatment in the NICU	46 (22.3)
Neonatal convulsions	12 (5.8)

CP – cerebral palsy; NICU – Neonatal Intensive Care Unit.
All values are expressed as numbers (percentages).

As indicated in Table 7, 22.3% of children with CP were treated in the Neonatal Intensive Care Unit (NICU), and 18.4% required assisted ventilation. Moreover, every sixth child with CP developed respiratory distress, hypoxic-ischemic encephalopathy was diagnosed in every ninth child, and neonatal convulsion in every seventeenth child with CP.

Discussion

CP is characterized by heterogeneous risk factors and specific etiology [14]. Although prematurity and low birthweight are the main risk factors for CP, it is also associated with congenital brain malformations, genetic predisposition, hypoxic-ischemic encephalopathy, stroke, kernicterus, maternal infection, multiple pregnancies, neonatal convulsions, neonatal or post-neonatal meningitis, and sepsis [1, 8, 14–16]. Yet, according to numerous epidemiological studies, half of the children with CP were carried to term and had none of the identified risk factors [1, 15–17].

In the cohort analyzed as a part of our study, hormonally maintained pregnancy, twin pregnancy, threatened abortion in the first half of pregnancy, bleeding after the 20th week of gestation, maternal infection, and anemia and hypertension in the second half of pregnancy were identified as the main prenatal risk factors for CP. As pregnant women are typically prescribed pharmacological therapy in order to prevent premature contractions and bleeding, the large percentage of hormonally maintained pregnancies, threatened abortions, and bleeding in our sample is not surprising. It is also worth noting that, according to Tollanes et al. [18], who studied birth outcomes in over 20,000 twins, the prevalence of CP is higher in twin pregnancies, indicating that CP in one twin increases the likelihood of CP in the other twin.

These findings concur with our data, as 28.9% of children with CP who had at least one of the prenatal risk factors were a result of twin pregnancies. The association between CP and twin pregnancy can be explained by a greater prevalence of premature birth in twins [5, 19]. In a population-based study conducted by Arnaud et al. [13] involving 2,273 premature babies, a quarter of the children were from multiple pregnancies. These authors noted that the presence of non-specific infection indicators, such as maternal fever, antibiotic use, and chorioamnionitis in the period immediately preceding labor, is associated with an increased risk of CP. This association has also been observed for maternal infections that occurred in early pregnancy, even though these findings

are inconclusive [8, 20]. In our study, every tenth mother reported having an infection during pregnancy, while in the sample analyzed by Elmagid and Magdy [5], this percentage was slightly lower (6%). Available evidence indicates that maternal infection, even in cases where there is no detectable inflammatory response, can lead to CP by transmitting the pathogen to the fetus and inducing systemic inflammation, which renders the developing brain sensitive to potential insults [21].

In our sample, prematurity was the leading perinatal risk factor, followed by low birthweight, low AS, assisted delivery (emergency cesarean section and vacuum fetal extraction), resuscitation, and pelvic presentation of the fetus. These results are in line with the available data, indicating that premature birth poses the greatest risk for the occurrence of CP, especially among extremely preterm babies [12–14, 22, 23]. For instance, about 10% of children born before 28 weeks of gestation are diagnosed with CP.

A meta-analysis of studies examining the relationship between CP prevalence and gestational age revealed that children born after 32−36, 28−31, and before 27 weeks of gestation had a 5−6, 32−54, and 60−130 times greater risk of developing CP in relation to children born at term, respectively [23]. In our cohort, prematurity was noted in about half of the cases, whereby 17% of children with CP were born after 28−31 weeks of gestation, and 7% were delivered before the 28th gestational week. Empirical evidence indicates that white matter damage as a result of intracerebral hemorrhage (ICH) and periventricular leukomalacia (PVL) is the most common pathological finding in premature infants who have developed CP [24, 25]. Moreover, Horber et al. [25] found that more than 80% of children born before 32 weeks of gestation had predominantly white matter damage, and its prevalence relative to gray matter damage was higher in this group of children compared to those born after 32 weeks of gestation. In extremely premature infants and those with extremely low birthweight, PVL and IVH are important prognostic factors for the development of CP [26]. The presence of infection also contributes to an increased risk of CP in premature infants [27, 28].

In our study, the prevalence of perinatal factors was high in children with CP delivered prematurely, which may be a consequence of the prenatal predisposition that led to premature birth. It is believed that children born prematurely are exposed to both perinatal and prenatal risk factors [29]. According to our data, perinatal risk factors are more prevalent in preterm infants than in those carried to term. This observation is in line with the available evidence suggesting that the number of children with at least one of the perinatal risk factors decreases significantly with gestational age, which may be associated with a gradual decline in the CP prevalence after 32 weeks of gestation [19, 26].

Our analyses further revealed that perinatal risk factors are most prevalent in the spastic bilateral type, followed by the dyskinetic type of CP. The bilateral spastic form of CP is most common in premature infants [6, 19, 30], and prematurity is recognized as the main risk factor in the perinatal period [31, 32]. Arnaud et al. [13] noted a bilateral spastic form of CP in two-thirds of preterm infants. According to our results, three-quarters of children with a diplegic form and half of the children with a quadriplegic form of CP were delivered prematurely. An ample body of evidence points to a link between premature birth and bilateral spastic diplegia [5, 30] as well as PVL occurrence [1, 15, 24, 25]. Spastic diplegia is typically attributed to damage to immature oligodendroglia during the 20–34 weeks of the gestational period, and PVL is the most common neuropathological substrate during neuroimaging [1, 15]. Spastic quadriplegia occurs in 20% of children with CP, and this clinical phenotype is also associated with premature birth and the presence of periventricular leukomalacia and multicystic cortical encephalomalacia [16].

Low AS, respiratory distress, and neonatal convulsions may be indicative of asphyxia but are also neurological signs that can indicate brain damage that occurred *in utero*. The role of asphyxia in the development of CP is related to the presence of other risk factors, such as markers of infection and placental disorders, which can result in abnormal fetal heart rate and low AS [33, 34]. In about 3% and 7% of cases analyzed as a part of our study, a placental abnormality and an AS < 7 were registered, respectively. However, when considered in isolation, these results have limited significance without determining the presence of other risk factors. According to Joud et al. [30], an AS < 7 which fails to improve after 5 min post-delivery is significantly associated with the development of CP.

Breech presentation at birth is thought to contribute to the development of asphyxia, but it may also be indicative of prenatal fetal abnormalities, such as intrauterine fetal growth retardation, fetal anomalies, oligohydramnios, gestational diabetes, and maternal thyroid dysfunction [35, 36]. In our study, 13.5% of children for whom data on the fetal position was available had breech presentations. Several authors have noted that assisted vaginal delivery or emergency cesarean section are significant risk factors for CP [5, 7, 13, 30]. Our analyses revealed that 41.4% of children with CP for whom the type of delivery was recorded required assisted childbirth, which is consistent with the findings based on a sample of 1,000 Egyptian children [5].

Based on our analyses, respiratory distress syndrome, the need for treatment in the NICU, assisted ventilation, hypoxic-ischemic encephalopathy (HIE), and neonatal convulsions emerged as the leading neonatal risk factors for CP. The first three factors noted above are causally related and are the focus of preventive measures, made possible by modern achievements in the field of intensive care and therapy that can be offered to newborns. Among the neonatal measures that can be adopted for the prevention of CP, induction of hypothermia in order to reduce intrapartum hypoxia and ischemia is particularly beneficial, according to some authors. In Sweden, this strategy was introduced in 2007 and has resulted in a significant reduction in the severe CP form [3, 9].

The lower prevalence of quadriplegic and dyskinetic forms of CP (considered a consequence of HIE) in the first decade of the 21st century compared to the last decade of the 20th century is also associated with recent advances in ob-

stetric and neonatal care [9]. Mechanical ventilation is widely used when caring for extremely premature babies and contributes to an increased risk of CP [37]. HIE during the neonatal period is a strong predictor of CP and occurs mainly in children born at term [5, 38]. Neonatal convulsions are early manifestations of brain damage and can be mild, reversible, or severe, whereby the latter form results in long-term disability. Together with a low AS or the presence of HIE, neonatal convulsions are thought to be indicative of perinatal asphyxia. However, there is a possibility that brain damage occurred earlier during pregnancy and was not associated with ischemia or hypoxia at birth [33].

Identifying specific risk factors for CP contributes to a better understanding of the potential mechanisms of pathogenesis. As different CP subtypes exhibit different etiologic spectra, they will inform the measures that can potentially be taken to address the preventable causes of CP, as well as the most optimal preventive strategies in these patients. Targeting the preventable risk factors could be crucial in modifying the CP trends.

Conclusion

According to our findings, hormonally maintained pregnancy, twin pregnancy, premature birth with low birthweight, assisted delivery, need for treatment in the NICU, and assisted ventilation are the most common risk factors for CP. However, most factors that increase the likelihood of CP do not act in isolation but rather lead synergistically to damage in the affected children. Perinatal risk factors are most prevalent in preterm infants and spastic bilateral CP type. Premature birth poses the greatest risk for the occurrence of CP, and spastic bilateral CP type was most prevalent in the group of preterm-born children included in our study.

REFERENCES

1. *Korzeniewski SJ, Slaughter J, Lenski M, Haak P, Paneth N.* The complex aetiology of cerebral palsy. Nat Rev Neurol 2018; 14(9): 528–43.
2. *Christensen D, Van Naarden Braun K, Doernberg NS, Maenner MJ, Arneson CL, Durkin MS,* et al. Prevalence of cerebral palsy, co-occurring autism spectrum disorders, and motor functioning - Autism and Developmental Disabilities Monitoring Network, USA, 2008. Dev Med Child Neurol 2014; 56(1): 59–65.
3. *Reid SM, Meehan E, McIntyre S, Goldsmith S, Badawi N, Reddihough D.* Temporal trends in cerebral palsy by impairment severity and birth gestation. Dev Med Child Neurol 2016; 58(Suppl 2): 25–35.
4. *Delobel-Ayoub M, Ehlinger V, Klapouszczak D, Maffre T, Raynaud JP, Delpierre C,* et al. Prevalence and characteristics of autism spectrum disorders in children with cerebral palsy. Dev Med Child Neurol 2017; 59(7): 738–42.
5. *Elmagid DSA, Magdy H.* Evaluation of risk factors for cerebral palsy. Egypt J Neurol Psychiatry Neurosurg 2021; 57: 13.
6. *Ikeudenta BA, Rutkofsky IH.* Unmasking the Enigma of Cerebral Palsy: A Traditional Review. Cureus 2020; 12(10): e11004.
7. *McIntyre S, Blair E, Badawi N, Keogh J, Nelson KB.* Antecedents of cerebral palsy and perinatal death in term and late preterm singletons. Obstetric Gynecol 2013; 122(4): 869–77.
8. *Yuan J, Wang J, Ma J, Zhu D, Zhang Z, Li J.* Paediatric cerebral palsy prevalence and high risk factors in Henan province, Central China. J Rehabil Med 2019; 51(1): 47–53.
9. *Hollung SJ, Vik T, Lydersen S, Bakken IJ, Andersen GL.* Decreasing prevalence and severity of cerebral palsy in Norway among children born 1999 to 2010 concomitant with improvements in perinatal health. Eur J Paediatr Neurol 2018; 22(5): 814–21.
10. *Patel DR, Neelakantan M, Pandher K, Merrick J.* Cerebral palsy in children: a clinical overview. Transl Pediatr 2020; 9(1): 125–35.
11. *Demeši Drljan C, Mikov A, Filipović K, Tomašević Todorović S, Knežević A, Krasnik R.* Cerebral palsy in preterm infants. Vojnosanit Pregl 2016; 73(4): 343–8.
12. *Sadowska M, Sarecka-Hujar B, Kopyta I.* Cerebral Palsy: Current Opinions an Definition, Epidemiology, Risk Factors, Classification and Treatment Options. Neuropsychiatr Dis Treat 2020; 16: 1505–18.
13. *Arnaud C, Ehlinger V, Delobel-Ayoub M, Klapouszczak D, Perra O, Hensey O,* et al. Trends in Prevalence and Severity of Pre/Perinatal Cerebral Palsy Among Children Born Preterm From 2004 to 2010: A SCPE Collaboration Study. Front Neurol 2021; 12: 624884.
14. *Himmelmann K, Ahlin K, Jacobsson B, Cans C, Thorsen P.* Risk factors for cerebral palsy in children born at term. Acta Obstet Gynecol Scand 2011; 90(10): 1070–81.
15. *Stavsky M, Mor O, Mastrolia SA, Greenbaum S, Than NG, Erez O.* Cerebral Palsy-Trends in Epidemiology and Recent Development in Prenatal Mechanisms of Disease, Treatment, and Prevention. Front Pediatr 2017; 5: 21.
16. *Graham HK, Rosenbaum P, Paneth N, Dan B, Lin JP, Damiano DL,* et al. Cerebral palsy. Nat Rev Dis Primers 2016; 2: 15082.
17. *Michael-Asalu A, Taylor G, Campbell H, Lelea LL, Kirby RS.* Cerebral palsy: diagnosis epidemiology, genetics and clinical update. Adv Pediatr 2019; 66: 189–208.
18. *Tollanes MC, Wilcox AJ, Lie RT, Moster D.* Familial risk of cerebral palsy: population based cohort study. BMJ 2014; 349: g4294.
19. *Maenner MJ, Benedict RE, Arneson CL, Yeargin-Allsopp M, Wingate MS, Kirby RS,* et al. Children with cerebral palsy: racial disparities in functional limitations. Epidemiology 2012; 23(1): 35–43.
20. *Brookfield KF, Osmundson SS, Caughey AB, Snowden JM.* Does Infection During Pregnancy Outside of the Time of Delivery Increase the Risk of Cerebral Palsy? Am J Perinatol 2017; 34(3): 223–8.
21. *Dammann O.* Causality, mosaics and the health science. Theor Med Biotech 2016; 37(2): 161–8.
22. *Schieve LA, Tian LH, Rankin K, Kogan MD, Yeargin-Allsopp M, Visser S,* et al. Population impact of preterm birth and low birth weight on developmental disabilities in US children. Ann Epidemiol 2016; 26(4): 267–74.
23. *Oskoui M, Coutinho F, Dykeman J, Jette N, Pringsheim T.* An update on prevalnce of cerebral palsy: a systematic review and meta-analysis. Dev Med Child Neurol 2013; 55(6): 509–19.
24. *Back SA.* White matter injury in the preterm infant: pathology and mechanisms. Acta Neuropathol 2017; 134(3): 331–49.
25. *Horber V, Sellier E, Horridge K, Rackauskatie G, Andersen GL, Virella D,* et al. The origin of the cerebral palsies: contribution of population based neuroimiging data. Neuropediatrics 2020; 51(2): 113–9.
26. *Linsell L, Malouf R, Morris J, Kurinczuk JJ, Marlow N.* Prognostic factors for cerebral palsy and motor impairment in children born very preterm or very low birthweight: a systematic review. Dev Med Child Neurol 2016; 58(6): 554–69.
27. *Kuban KC, O'Shea TM, Allred EN, Paneth N, Hirtz D, Fichorova RN,* et al. ELGAN Study Investigators. Systemic inflammation

and cerebral palsy risk in extremely preterm infants. J Child Neurol 2014; 29(12): 1692–8.
28. *Kuban KC, O Shea TM, Allred E, Fichorova RN, Heeren T, Paneth N,* et al. The breath and type of systemic inflammation and the risk of adverse neurological outcomes in extremely low gestation newborns. Pediatr Neurol 2015; 52(1): 42–8.
29. *Stoknes M, Andersen GL, Elkamil AI, Irgens LM, Skranes J, Salvensen KA*, et al. The effects of multiple pre- and perinatal risk factors on the occurence of cerebral palsy. A Norwegian register based study. Eur J Paediatr Neurol 2012; 16(1): 56–63.
30. *Joud A, Sehlstedt A, Kallen K, Westom L, Rylander L.* Association between antenatal and perinatal risk factors and cerebral palsy: a Swedish cohort study. BMJ Open 2020; 10(8): e038453.
31. *Pascal A, Govaert P, Oostra A, Naulers G, Ortibus E, Van den Broeck C.* Neurodevelopmental outcome in very preterm and very low-birthweight infants born over the past decade: a meta analytic review. Dev Med Child Neurol 2018; 60: 342–55.
32. *Hafsrom M, Kallen K, Serenius F, Marsal K, Rehn E, Drake H*, et al. Cerebral palsy in extremely preterm infants. Pediatrics 2018; 141: e20171433.
33. *Ellenberg JH, Nelson KB.* The association of cerebral palsy with birth asphyxia: a definitional quagmire. Dev Med Child Neurol 2013; 55(3): 210–6.
34. *Nelson KB, Sartwelle TP, Rouse DJ.* Electronic fetal monitoring, cerebral palsy, and caesarean section: assumptions versus evidence. BMJ 2016; 355: i6405.
35. *Vissenberg R, Vrijkotte TG, van der Post JA, Fliers E, Goddijn M, Bisschop PH.* Abnormal thyroid function parameters in the second trimester of pregnancy are associated with breech presentation at term: a nested cohort study. Eur J Obstet Gynecol Reprod Biol 2016; 199: 169–74.
36. *Macharey G, Gissler M, Rahkonen L, Ulander VM, Väisänen-Tommiska M, Nuutila M,* et al. Breech presentation at term and associated obstetric risks factors-a nationwide population based cohort study. Arch Gynecol Obstet 2017; 295(4): 833–8.
37. *Tsai WH, Hwang YS, Hung TY, Weng SF, Lin SJ, Chang WT.* Association between mechanical ventilation and neurodevelopmental disorders in a nationwide cohort of extremely low birth weight infants. Res Dev Disabil 2014; 35(7): 1544–50.
38. *Badawi N, Felix JF, Kurinczuk JJ, Dixon G, Watson L, Keogh JM,* et al. Cerebral palsy following term newborn encephalopathy: a population-based study. Dev Med Child Neurol 2005; 47(5): 293–8.

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Comparative analysis of stress and deformation distribution in implant-supported telescopic systems made of different materials

Uporedna analiza distribucije pritiska i deformacije kod implantno-nošenih teleskop sistema izrađenih od različitih materijala

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Abstract

Background/Aim. In implant prosthetics, there is an increasing use of materials that, with their mechanical characteristics, can alleviate the negative consequences of implant stress. The aim of this study was to conduct a comparative analysis of stress distribution and deformation of implant-supported telescopic systems and surrounding structures made of different materials using the finite element method. **Methods.** The 3D finite element models were prepared using the SolidWorks program (SolidWorks 2018, Concord, MA, USA). Two models of telescopic crowns with the characteristics of polyetheretherketone (PEEK) polymer and cobalt-chromium (Co-Cr) alloy faceted with feldspar ceramics were used. The models were loaded with an axial force of 150 N in the region of the central *fossa*. The analysis of stress and strain distribution was performed by the finite element method in the Ansys software (ANSYS Workbench 16; Ansys Inc., Pittsburg, PA, USA). **Results.** Implant-supported telescopic crowns made of PEEK polymer significantly reduced stress in the implant and abutment neck area compared to the conventional Co-Cr crown veneered ceramic. At the level of bone structure, both models showed a concentration of stress at the level of the cortical bone, while the trabecular bone was significantly less exposed to stress. Under the same conditions, the degree of deformation of the secondary telescopic crown was more pronounced in models with PEEK polymer characteristics. **Conclusion.** Owing to their mechanical characteristics, PEEK polymers can be the materials of choice in the fabrication of superstructures on implants. Given that this *in vitro* study was accompanied by limitations, further research is needed to confirm the superior role of PEEK material in implant prosthetics.

Key words:
cobalt; chromium; computer-aided design; crowns; dental materials; dental stress analysis; polymers.

Apstrakt

Uvod/Cilj. U implantoprotetici se sve više koriste materijali koji svojim mehaničkim karakteristikama mogu ublažiti negativne posledice pritiska implantata. Cilj rada bio je da se sprovede uporedna analiza distribucije pritiska i deformacije implantno-nošenih teleskop kruna i okolnih struktura, izrađenih od različitih materijala, korišćenjem metode konačnih elemenata. **Metode**. Korišćenjem programa SolidWorks (SolidWorks 2018, Concord, MA, USA) pripremljeni su 3D modeli konačnih elemenata. Korišćena su dva modela teleskop kruna sa karakteristikama polietereterketon (PEEK) polimera i kobalt-hrom (Co-Cr) legure, fasetirane keramikom od feldspara. Modeli su bili opterećeni aksijalnom silom od 150 N u predelu centralne fose. Analiza distribucije pritiska i deformacije sprovedena je metodom konačnih elemenata u Ansys programu (ANSYS Workbench 16; Ansys Inc., Pittsburg, PA, USA). **Rezultati**. Implantno-nošene teleskop krune izrađene od PEEK polimera značajno su smanjivale pritisak u zoni vrata implantata i suprastrukture u poređenju sa konvencionalnom Co-Cr krunom fasetiranom keramikom. Na nivou koštane strukture, oba modela pokazala su koncentraciju pritiska na nivou kortikalne kosti, dok je trabekularna kost bila značajno manje izložena pritisku. Pri istim uslovima, stepen nastale deformacije sekundarne teleskop krune bio je viši kod modela sa karakteristikama PEEK polimera. **Zaključak**. Zahvaljujući mehaničkim karakteristikama, PEEK polimeri mogu biti materijali izbora u izradi suprakonstrukcija na implantatima. Kako je prezentovana *in vitro* studija praćena ograničenjima, neophodna su dalja istraživanja koja bi potvrdila superiornu ulogu PEEK materijala u implantoprotetici.

Ključne reči:
kobalt; hrom; kompjuterski podržan dizajn; zub, kruna; stomatološki materijali; stomatološki stres, analiza; polimeri.

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Introduction

The telescopic crowns represent the culmination of biological and esthetic prosthetic rehabilitation. In some circumstances, depending on the layout of the carrier and the degree of resorption of bone tissue, as well as the possibility of later repair and proper hygiene, the telescopic crown is the only therapeutic solution. Owing to some advantages, telescopic systems extend the life of abutment teeth compared to other prosthetic restorations [1]. The telescope system consists of an inner (primary) and outer (secondary) crown. Classical cylindrical telescopes function on the principle of friction, although the jamming effect, which occurs with small movements of the prosthesis, also plays an important role in retention [2].

Three double crown systems are used in implant prosthetics: classical telescopic crowns, galvanic telescopes, and telescopes with additional retention elements. Some authors recommend that the primary crowns of implant-supported double crowns be slightly conical (1 °–2 °) to avoid imprinting and laboratory errors, which can make it difficult to place and remove the supraconstruction [3]. These intraoral inaccuracies can lead to a feeling of discomfort in the patient and an increase in stress at the implant level. In the case of taking care of telescopes whose carriers are natural teeth, due to the orthodontic movement of the same, the discomfort disappears after a few days. However, this compensatory mechanism is lacking in osseointegrated implants, which cannot be moved orthodontically and could result in permanent patient discomfort and relatively rapid implant loss [2].

In implant prosthetics, the use of materials that can alleviate the negative consequences of implant stress with their mechanical characteristics and provide comfort to patients is increasing. In that sense, polyetheretherketone (PEEK) polymers are being increasingly used due to their mechanical and biological characteristics. PEEK materials are basically semicrystalline linear polycyclic aromatic polymers. Young's modulus of elasticity and tensile properties are close to human bone, enamel, and dentin. At the same time, PEEK polymer is resistant to various nontoxic and biocompatible chemical agents [4]. It is stable at high temperatures (during the sterilization process) and resistant to wear [5].

Analysis of the influence of mechanical characteristics of materials on the distribution of stress on individual intraoral structures *in vitro* is often expensive and time-consuming [6, 7]. One of the methods that can supplement or replace such research is a computer simulation, such as the finite element method (FEM).

The aim of this study was to conduct a comparative analysis of stress distribution and deformation of the secondary crown, implants, and surrounding bone in implant-supported telescopic crowns made of PEEK polymer and cobalt-chromium (Co-Cr) alloy veneered with ceramics using the FEM.

Methods

The first step in the research was the formation of 3D models necessary for the analysis. Using conical beam computed tomography (CBCT), a 3D image of the lower jaw in the region of the second premolar on the right side was made. The cross-section in the transverse plane was analyzed, and the contour of the bone cross-section of that region was reconstructed using the Corel draw vector graphics program. The resulting image was then extruded in the z-axis, using the appropriate Fusion 360-Autodesk program, creating a 3D model of the mandibular segment with a mesiodistal diameter of 10 mm. The bone was modeled so that the trabecular bone formed a nucleus surrounded by a layer of compacta. The dimensions of the trabecular bone were 9 mm in the laterolateral direction and 14 mm in the craniocaudal direction. The thickness of the compact part of the bone averaged 1.5 mm.

The one-piece dental implant model was designed using the program SolidWorks 2018 (Concord, MA, USA). The dimensions of the implant were 14.5 × 5 mm, with a platform height of 1.5 mm, a thread pitch of 0.9 mm, and a depth of 0.2 mm. The analysis was focused on the secondary crown and, therefore, the abutment and the implant were combined into one whole. After that, the one-part model of the implant was processed in the SolidWorks program in which the virtual abutment milling was performed at an angle of 90 °.

Based on the abutment, a primary crown telescope 3 mm wide and 5 mm high was formed, with a half-groove width of 1 mm. Then, the virtual implantation of a one-piece model of the implant into a previously designed bone model was performed.

The secondary crown model was obtained by scanning the real model. Based on the obtained scan by reverse engineering, a solid model was created using the SolidWorks 2018 program. In the same program, the inside of the secondary crown was formed using the Cut option in order to make it congruent with the outer surface of the primary telescopic crown of the implant. On average, the crown was 9 mm high, 10.9 mm wide, and 3 mm thick.

Two experimental models were used in this study. The characteristics of a PEEK polymer were given to the first model (Figure 1a). In the case of the second model, the secondary crown was given the characteristics of Co-Cr alloy, 1 mm thick, faceted with feldspar ceramics to the final morphological shape (Figure 1b).

Fig. 1 − Experimental models: a) Model with polyetheretherketone (PEEK) characteristics; b) Model with cobalt-chromium alloy characteristics.

Mechanical characteristics of materials

All materials used in the study were considered homogeneous, linear, and isotropic. The mechanical properties of the materials used in the study were collected from the published literature [8–15] (Table 1). Young's modulus, Poisson's ratio, and density were used as material characteristics. Based on these features, a library of materials in the Ansys program was created, which was later used during the FEM analysis. The study was conducted under the assumption that the implant was completely osseointegrated.

Loads and limitations

A linear static structural simulation was performed using ANSYS Workbench 16.0 (Ansys, Inc.). It shows the relationship (deformation and stress) between the secondary telescopic crown, the implant-abutment, and the bone. The finite element models in the first experimental model consisted of 93,463 triangular elements and 159,100 nodes, while the second experimental model consisted of 97,985 triangular elements and 167,170 nodes. In this study, the implant was subjected to an axial static load of 150 N with an attack point of force in the immediate vicinity of the central *fossa* [16].

The contact conditions between the components of each model are clearly defined, with the bonded type of connection being mostly represented. The contact between the primary and secondary crown was defined as the frictional ratio, with a friction coefficient of 0.2 k (Tables 2 and 3).

Results

Analysis of von Mises stress and deformation values of the secondary crowns

After the analysis, it was noticed that the stress concentration of the secondary crown in the first model was located in the region of action of the attacking force, i.e., the zone of the central *fossa*, while in the second model, the highest stress concentration was in the zone of the marginal line. It was also notable that these stress values were somewhat lower in the secondary crown made of PEEK polymer compared to the secondary crown made of Co-Cr veneered with porcelain.

Table 1

Mechanical properties of tested materials

Materials	Young's modulus (MPa)	Poisson's ratio	Density (g/cm^3)
Cortical bone	13,700	0.3	1.85
Trabecular bone	1,370	0.3	0.9
Ti-6Al-4V implant	110,000	0.35	4.51
Co-Cr alloy	218,000	0.33	10
Feldspar porcelain	65,000	0.25	2.45
PEEK Juvora	5,591	0.36	1.3

Ti-6Al-4V – titanium-aluminium-vanadium; Co-Cr – cobalt-chromium; PEEK – polyetheretherketone; MPa – megapascal.

Table 2

Contact between components in the first model

Cortical bone	Trabecular bone	Bonded
Cortical bone	implant	bonded
Trabecular bone	implant	bonded
Abutment	secondary crown	frictional

Table 3

Contact between components in the second model

Cortical bone	Trabecular bone	Bonded
Cortical bone	implant	bonded
Trabecular bone	implant	bonded
Abutment	secondary crown (Co-Cr)	frictional
Secondary crown (Co-Cr)	feldspar ceramics	bonded

Co-Cr – cobalt-chromium.

The axial load was performed next to the central *fossa* of the secondary crown. Figure 2 shows the behavior of a secondary crown made of PEEK polymer at an axial load of 150 N. By analyzing the results of stress, it can be said that its highest concentration was localized on the outer surface of the secondary crown itself near the attack point of force. Most of the stress was amortized by the secondary crown or its surface layers. Therefore, the inner surface of the secondary crown was in the zone of minimal stress. That also reduced the transmission of stress to the implant (Figure 2a).

In the second model, the highest concentration of stress was localized in the zone of the marginal line, i.e., at the edge of the secondary crown (Figure 2b). It was also noticeable that these stress values were slightly higher in the secondary crown made of PEEK polymer compared to the secondary crown made of Co-Cr faceted with porcelain.

When it comes to deformation, it was most pronounced in both models in the region of action of the attacking force (Figure 3a). From the aspect of deformation intensity, higher values were present in the first model, which is in line with the lower values of the modulus of elasticity of the PEEK polymer (Figure 3 b).

Analysis of von Mises stress and deformation values of the implant model

At the implant level, in both examined models, the highest stress concentration can be seen in the area of the implant neck. However, the stress values differ significantly in the first and second models. In the first model with a secondary crown made of PEEK polymer, the amount of stress was almost twice as low as in the second model with a secondary crown made of Co-Cr alloy veneered with ceramics (Figures 4a and 4b). From the aspect of deformation, it was somewhat more pronounced in the first model (Figures 5a and 5b).

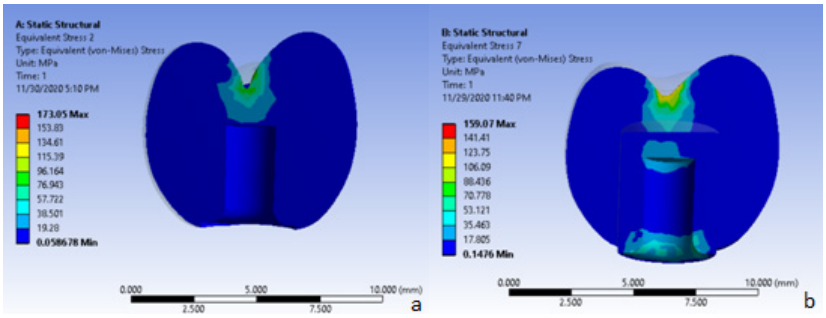

Fig. 2 − Sagittal section views for stress distribution of crown: a) First experimental model; b) Second experimental model.

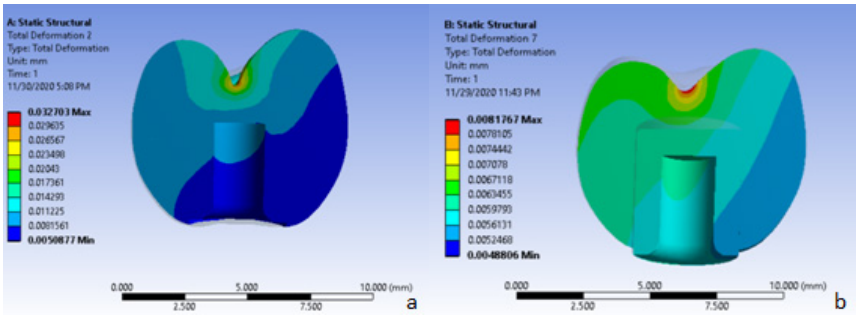

Fig. 3 − Sagittal section views for deformation of the crown: a) First experimental model; b) Second experimental model.

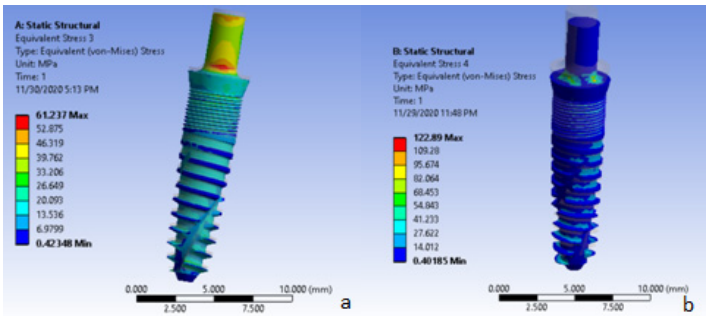

Fig. 4 − Stress distribution of implant: a) First experimental model; b) Second experimental model.

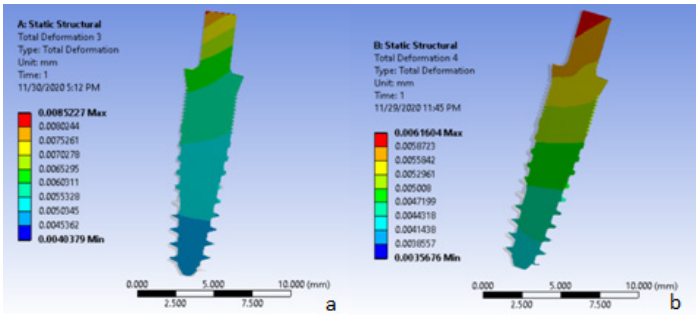

Fig. 5 − Sagittal section views for deformation of implant: a) First experimental model; b) Second experimental model.

Analysis of von Mises stress and deformation values of the bone

Analysis of stress at the level of bone tissue showed that most of the stress is accepted by the cortical bone next to the implant neck. At the same time, significant differences in stress values were not observed between the experimental models (Figure 6). Deformation of the cortical bone does not show significant differences in both examined models (Figure 7).

At the level of trabecular bone stress, intensity is significantly lower than in cortical bone. Such findings are a consequence of the acceptance of most of the stress by the cortical bone, and a smaller amount of stress is transferred to the trabecular bone. However, the analysis of stress values at the level of the spongy bone showed significantly lower values in the first experimental model, where the PEEK secondary telescopic crown was used (Figure 8). The analysis of spongy bone deformation does not show significant differences between the examined models (Figure 9).

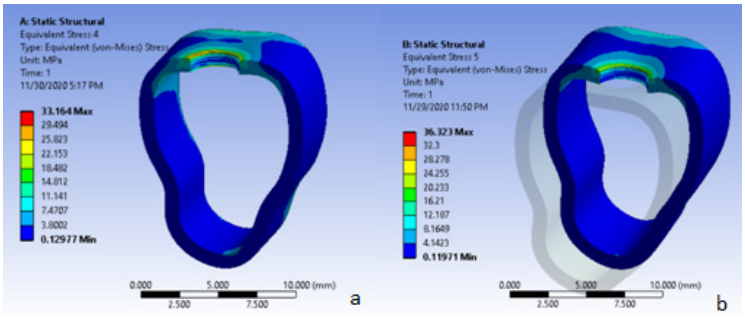

Fig. 6 − Sagittal section views for stress distribution of cortical bone: a) First experimental model; b) Second experimental model.

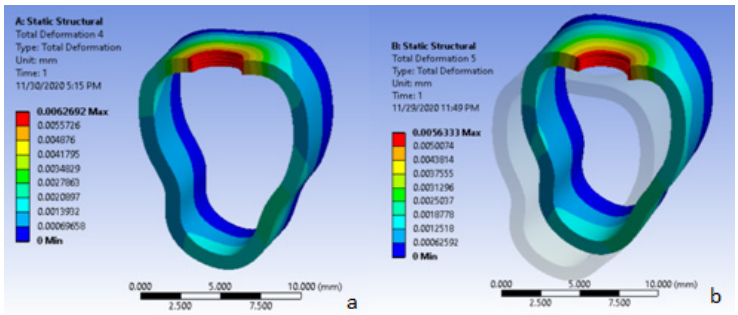

Fig. 7 − Sagittal section views for deformation of cortical bone: a) First experimental model; b) Second experimental model.

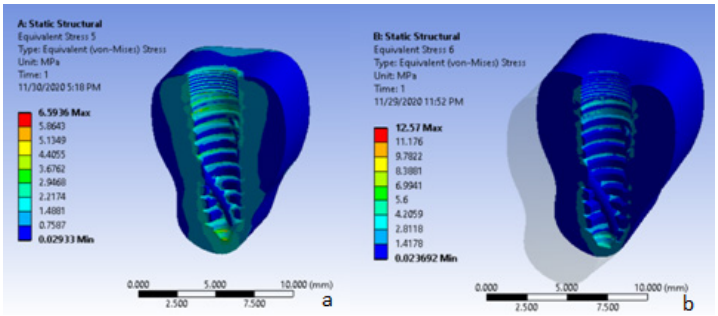

Fig. 8 − Sagittal section views for stress distribution of spongy bone: a) Frst experimental model; b) Second experimental model.

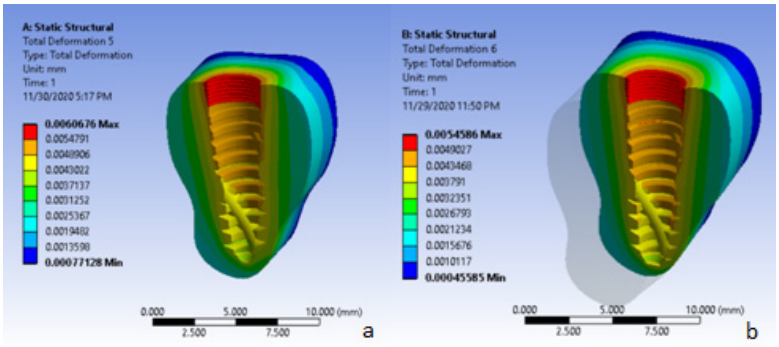

Fig. 9 − Sagittal section views for deformation of spongy bone: a) First experimental model; b) Second experimental model.

Discussion

Tooth loss unequivocally requires prosthetic rehabilitation of the patient. It is believed that only properly performed prosthetic therapy can reduce the bone resorption that inevitably occurs after tooth loss [17]. For these reasons, this study aimed to examine the intensity of stress on the secondary telescopic crown, implant, and bone tissue, which develops when using different materials to make superstructures on implants. The research was conducted under the assumption that the used models were homogeneous, isotropic, and linearly elastic. However, it is known that there is no absolutely homogeneous and isotropic material in nature, so the use of mean values does not exclude the possibility of errors in the results of *in vitro* tests [18]. These facts represent some of the limiting factors of this study. In this study, an occlusal axial load of 150 N was used, which is the average value of the worrying forces produced in patients with implants [19]. However, *in vitro* conditions during the function of the stomatognathic system also develop extra-axial forces that can have a more detrimental effect on implants and prosthetic restorations. Accordingly, this could also be a limiting factor in this research.

When it comes to telescopes on implants, the frictional ratio of the primary and secondary crown gives additional freedom of movement, which reduces the stress on the implant itself. Some studies have confirmed that stress and cortical bone deformities are significantly less in implant-supported telescopic crowns than in certain superstructures on locators [20].

A special focus of this research was on PEEK polymer, which is more biocompatible and lighter than metal, so it represents its suitable alternative. Furthermore, PEEK polymer does not cause galvanic corrosion if it comes in contact with other metals in the mouth [21]. The results of our research indicate that secondary crowns made of PEEK provide significant stress adsorption and protection of surrounding structures from stress. These findings can be explained by a similar modulus of elasticity between the PEEK polymer and the bone structure, resulting in less stress on the bone and implant.

In a similar study, Tekin et al. [22] analyzed the distribution of stress and strain in fixed restorations made of PEEK polymers. The results of this study showed that the modulus of elasticity of PEEK material and bone is similar and that, in this way, the incoming forces are absorbed, and the stresses on the bone structures are minimized. It was also found that von Mises stresses on the PEEK crown were concentrated at the marginal end line, but the stress value was reduced at the abutment level. The results of our research show that at the level of implants, in both examined models, the highest concentration of stress is present in the area of the implant neck. At the same time, the value of stress was twice lower in the PEEK model compared to the model of Co-Cr alloy veneered with ceramic. The local concentration of stress on the secondary PEEK telescopic crown and its deformation reduce the transmission of stress to the primary crown and abutment. These findings indicate that the PEEK polymer can act as a stress absorber, protecting the surrounding structures from excessive stress. Zoidis and Papathanasiou [23] found that the PEEK crown did not make a significant difference in relation to the metal-ceramic one in terms of stress on bones and implants but that the use of the PEEK crown certainly reduced the stress on the abutment. Dashti et al. [24] found that PEEK crowns reduce stress on the abutment, as well as that the highest values of stress are observed in the zone of cortical bone around the neck of the implant. This fact indicates the adsorption of stress by the cortical bone and the reduction of stress transmission to the trabecular bone. The findings of this study support the results of our research.

El-Anwar et al. [25] concluded that the material from which the crown was made has a negligible effect on the distribution of forces on the cortical bone. Our study also showed that the values of stress that occur on the cortical bone during loading are very similar in both tested models.

Studies have shown that PEEK softens the effects of masticatory forces precisely because of its elasticity [26, 27]. The elasticity of this material is especially important in prosthetic restorations that are implant-worn, where, due to the lack of mechanoreceptors of the periodontium, the control of mastication is reduced in the absence of inhibitory mechanisms. Therefore, stress is more present in implant-compensated restorations than in natural dentition. Our research, along with available data from the literature, indicates that PEEK polymers will be a good alternative to metal alloys in the future.

Conclusion

PEEK polymers reduce the distribution of stress at the level of implants, abutments, and trabecular bone. Owing to their mechanical characteristics, PEEK polymers can be the materials of choice in the fabrication of superstructures on implants. However, because this *in vitro* study has some limitations, further research is needed to confirm the superior role of PEEK material in implant prosthetics.

REFERENCES

1. *Korberg K.* Konuskronen – telescope: Einfuhrung in Klinik und Technik. Heindelberg: Huthig; 1973. (German)
2. *Wolfart S.* Implantoprosthetics: A patient-centered concept. Croatia, Zagreb: Quintessence publishing; 2015. (Croatian)
3. *Diedrichs G, Rosenhain P*, Galvano-Außenteleskope in der direkten Technik. Quintessenz 1991; 42 (49): 49–55. (German)
4. *Garcia-Gonzalez D, Rusinek A, Jankowiak T*, *Arias A.* Mechanical impact behavior of polyether-ether-ketone (PEEK). Compos Struct 2015; 124: 88–99.
5. *Zhou L, Qia Y, Zhu Y, Liu H, Gan K, Guo J.* The effect of different surface treatments on the bond strength REFERENCES of PEEK composite materials. Dent Mater 2014; 30(8): e209–15.
6. *Farah WJ, Craig GR, Meroueh KA.* Finite element analysis of a mandibular model. J Oral R 1988; 15(6): 615–24.
7. *Darendeliler S, Darendeliler H, Kinoğlu T.* Analysis of a central maxillary incisor by using a three-dimensional finite element method. J Oral Rehabil 1992; 19(4): 371–83.
8. *Cervino G, Romeo U, Lauritano F, Bramanti E, Fiorillo L, D'Amico C,* et al. Von Mises Analysis of OSSTEM ® Dental Implant Structural Components: Evaluation of Different Direction Dynamic Loads. Open Dent J 2018; 12: 219–29.
9. *Pessoa RS, Coelho PG, Muraru L, Marcantonio E Jr, Vaz LG, Vander Sloten J,* et al. Influence of implant design on the biomechanical environment of immediately placed implants: computed tomography-based nonlinear three-dimensional finite element analysis. Int J Oral Maxillofac Implants 2011; 26(6): 1279–87.
10. *Ding X, Liao SH, Zhu XH, Zhang XH, Zhang L.* Effect of diameter and length on stress distribution of the alveolar crest around immediate loading implants. Clin Implant Dent Relat Res 2009; 11(4): 279–87.
11. *Alvarez-Arenal A, Segura-Mori L, Gonzalez-Gonzalez I, Gago A.* Stress distribution in the abutment and retention screw of a single implant supporting a prosthesis with platform switching. Int J Oral Maxillofac Implants 2013; 28(3): e112–21.
12. *Bona AD, Anusavice KJ, DeHoff PH.* Weibull analysis and flexural strength of hot-pressed core and veneered ceramic structures. Dent Mater 2003; 19(7): 662–9.
13. *Tekin S, Adıgüzel Ö, Cangül S.* An evaluation using micro-CT data of the stress formed in the crown and periodontal tissues from the use of PEEK post and PEEK crown: A 3D finite element analysis study. Int Dent Res 2018; 8(3): 144–50.
14. *Siewert B, Parra M,* A new group of maretial in dentistry. PEEK as a framework material for 12piece implant supported bridges. Zahnarztl Implantol 2013; 29: 148–59.
15. *Muhsin SA, Hatton PV, Johnson A, Sereno N, Wood DJ.* Determination of Polyetheretherketone (PEEK) mechanical properties as a denture material. Saudi Dent J 2019; 31(3): 382–91.
16. *Geramizadeh M, Katoozian H, Amid R, Kadkhodazadeh M.* Static, Dynamic, and Fatigue Finite Element Analysis of Dental Implants with Different Thread Designs. J Long Term Eff Med Implants 2016; 26(4): 347–55.
17. *Samra RK, Bhide SV, Goyal C, Kaur T.* Tooth supported overdenture. A concept overshadowed but not yet forgotten! J Oral Res Rev 2015; 7(1): 16–21.
18. *Meijer HJ, Starmans FJ, Bosman F, Steen WH.* A comparison of three finite element models of an edentulous mandible provided with implants. J Oral Rehabil 1993; 20(2): 147–57.
19. *Brunski JB, Puleo DA, Nanci A.* Biomaterials and biomechanics of oral and maxillofacial implants: current status and future developments. Int J Oral Maxillofac Implants 2000; 15(1): 15–46.
20. *Abbasi MRA, Vinnakota DN, Sankar V, Kamatham R.* Comparison of stress induced in mandible around an implant-supported overdenture with locator attachment and telescopic crowns - a finite element analysis. Med Pharm Rep 2020; 93(2): 181–9.
21. *Najeeb S, Zafar MS, Khurshid Z, Siddiqui F.* Applications of polyetheretherketone (PEEK) in oral implantology and prosthodontics. J Prosthodont Res 2016; 60(1): 12–9.
22. *Tekin S, Değer Y, Demirci F.* Evaluation of the use of PEEK material in implant-supported fixed restorations by finite element analysis. Niger J Clin Pract 2019; 22(9): 1252–8.
23. *Zoidis P, Papathanasiou I.* Modified PEEK resin-bonded fixed dental prosthesis as an interim restoration after implant placement. J Prosthet Dent 2016; 116: 637–41.
24. *Dashti MH, Atashrazm P, Emadi MI, Mishaeel S, Banava S.* The effects of two attachment types on the stresses introduced to the mandibular residual ridge: a 3D finite element analysis. Quintessence Int 2013; 44(8): 585–90.
25. *El-Anwar MI, El-Mofty MS, Awad AH, El-Sheikh SA, El-Zawahry MM.* The effect of using different crown and implant materials on bone stress distribution: a finite element study. Egypt J Oral Maxillofac Surg 2014; 5(2): 58–64.
26. *Von Meyer H.* Die architectur der spongiosa. Archiv fur Anatomie und Physiologie 1867; 47: 615–28. (German)
27. *Stephan A, Steffen K, Frank K, Jörg L, Jörg N.* A wealth of possible applications for high-performance polymers. Quintessenz Zahntech 2013; 39: 2–10.

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Laser peripheral iridotomy in patients with acute primary angle closure

Laserska periferna iridotomija kod bolesnika sa akutnim primarno zatvorenim komornim uglom

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Abstract

Background/Aim. Since glaucoma has a very high prevalence worldwide, it is important to examine additional treatment modalities, especially the prevention of its progression. The aim of the study was to determine the importance of laser peripheral iridotomy (LPI) in patients with acute primary angle closure (APAC) in preventing primary angle closure glaucoma progression and APAC in the fellow eye. **Methods.** The research included 40 patients and 80 eyes treated between 2017 and 2021, which was also the follow-up period in the study. In all patients, LPI was performed bilaterally on both the APAC-affected eye and the healthy fellow eye. The patients with an age range from 40 to 79 years who had the pupillary block in one eye were monitored. All patients underwent bilateral LPI, and the changes in angle width were monitored using gonioscopy. Intraocular pressure (IOP) measurements were made with an applanation tonometer and Vertical Cup/Disc ratio (Ver C/D rat) performing biomicroscopic examination with indirect ophthalmoscopy and +90 D lens. **Results.** All 40 patients underwent bilateral LPI. The angle width of the APAC-affected eye before treatment was 0.15 ± 0.36, and 1.20 ± 0.41 of the fellow eye. After 12 months, the measurements taken were 0.85 ± 0.36 for the affected eye and 1.90 ± 0.36 for the fellow eye (Wilcoxon rank test, $p < 0.01$ statistically significant difference). The mean value of IOP in the eye without progression of the disease before therapy was 53.6 ± 3.73 mmHg, while in the eye with progression, it was 60.10 ± 4.37 mmHg. After 12 months, it was 14.92 ± 1.22 mmHg in the eye without progression, while in the eye with disease progression, it was 23.40 ± 2.53 mmHg (independent samples *t*-test, $p < 0.01$). The change in the Ver C/D rat in the eye without progression was 0.40 ± 0.10, while in the eye with progression, it was 0.45 ± 0.05. After 12 months, it remained unchanged in the eye without progression, while in the eye with progression, it was 0.65 ± 0.06 (independent samples *t*-test, $p < 0.01$). **Conclusion.** Simultaneous LPI has been proven efficient in patients with APAC in both affected and fellow eyes.

Key words:
glaucoma, angle closure; laser therapy; ophthalmologic surgical procedures.

Apstrakt

Uvod/Cilj. Glaukom je veoma rasprostranjen širom sveta zbog čega je važno ispitati dodatne načine lečenja osoba sa glaukomom, a posebno prevenciju njegove progresije. Cilj rada bio je da se utvrdi značaj laserske periferne iridotomije (LPI) u lečenju bolesnika sa akutnim primarno zatvorenim komornim uglom (APZK) u prevenciji progresije u primarni glaukom zatvorenog ugla, kao i pojavi APZK na parnom oku. **Metode.** Istraživanjem je obuhvaćeno 40 bolesnika, 80 očiju, lečenih u periodu od 2017. do 2021. godine, što je ujedno bio i period praćenja u studiji. Kod svakog bolesnika rađena je LPI obostrano - i na oku sa APZK i na parnom zdravom oku. Praćeni su bolesnici starosti 40–79 godina, koji su imali blokadu zenice u jednom oku. Kod svih bolesnika urađena je LPI na oba oka i praćene su promene širine komornog ugla gonioskopijom. Intraokularni pritisak (IOP) je meren aplanacionom tonometrijom i *Vertical Cup/Disc ratio (Ver C/D rat)* indirektnom oftalmoskopijom na biomikroskopu, korišćenjem lupe od +90 D. **Rezultati.** Kod svih 40 bolesnika urađena je LPI na oba oka. Širina komornog ugla na oku sa APZK pre terapije iznosila je 0,15 ± 0,36, a na parnom oku 1,20 ± 0,41. Nakon 12 meseci na oku sa APZK izmerena širina komornog ugla je iznosila 0,85 ± 0,36, a na parnom oku 1,90 ± 0,36 (Wilcoxon test ranga, $p < 0,01$ statistički značajna razlika). Prosečna vrednost IOP na oku bez progresije bolesti pre terapije bila je 53,61 ± 3,73 mmHg, na oku sa progresijom bolesti 60,10

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± 4,37 mmHg. Nakon 12 meseci na oku bez progresije izmerena vrednost bila je 14,92 ± 1,22 mmHg, a na oku sa progresijom 23,40 ± 2,53 mmHg (*t*-test za nezavisne uzorke, $p < 0,01$). Promena *Ver C/D rat* na oku bez progresije bila je 0,40 ± 0,10, na oku sa progresijom 0,45 ± 0,05, a nakon 12 meseci na oku bez progresije ostala je nepromenjena, dok je na oku sa progresijom bila 0,65 ± 0,06 (*t*-test za nezavisne uzorke, $p < 0,01$). **Zaključak.** Istovremena primena LPI bila je efikasna u lečenju bolesnika sa APZK i na bolesnom i na parnom oku.

Ključne reči:
glaukom, zatvorenog ugla; lečenje laserom; hirurgija, oftalmološka, procedure.

Introduction

Glaucoma is the most common neurodegenerative disease leading to structural and functional changes in the optic nerve. It affects 80 million people worldwide. It is the second cause of blindness [1, 2]. Considering eyesight, primary angle-closure glaucoma (PACG) is far more destructive than open-angle glaucoma and affects 20 million people worldwide [3]. Acute primary angle closure (APAC) is defined as the presence of contact between the iris and trabecular meshwork as well as the possible presence of peripheral anterior synechiae (PAS), which leads to elevated intraocular pressure (IOP) without changes in the optic nerve head [4]. The incidence of acute angle closure is the highest in Singapore, occurring annually at 12.2 per 100,000 people over 30 years of age [5]. The risk of its occurrence is a narrow angle (≤ 20°) [4]. In 75% of cases, the pupillary block is the reason for its occurrence [6]. Initial medication treatment is administered to prepare patients for LPI, which should be performed as soon as possible in all patients with APAC to remove the pupillary block and as a preventive measure for the fellow eye [7, 8]. If IOP cannot be controlled, additional surgical treatment such as lens extraction or trabeculectomy (TTR) is indicated [9]. LPI is the standard treatment of PACG, as well as the preventive treatment of APAC [10–12]. Moreover, it reduces the risk of acute attack in the fellow eye [13–16].

The aim of the study was to present the importance of LPI in patients with APAC preventing its progression to PACG and the occurrence of APAC in the fellow eyes.

Methods

The research was conducted as a retrospective cohort interventional study, with the participants being both the study subjects and controls. The research included 40 patients who suffered unilateral APAC due to pupillary block aged 40 to 79 years, admitted to the Clinic of Ophthalmology, University Clinical Center in Kragujevac, Serbia. The patients did not have glaucoma or glaucomatous optic neuropathy (GON) before APAC. All patients were monitored over 12 months after LPI, as presented in the research. The research was performed from 2017 to 2021, and it was approved by the Research Ethics Committee of the University Clinical Center in Kragujevac (No. 01-8678 from September 27, 2010). After hospital admission, all patients were first treated with systemic and local drugs to achieve a reduction in IOP and improve corneal transparency, after which LPI was performed in the affected eye, and then a few days after, during hospitalization, the patients underwent prophylactic LPI in the healthy fellow eye. In all patients, the LPI procedure was performed using Nd:YAG laser (Carl Zeiss, Germany). The laser energy used during LPI ranged from 1.8 to 4.1 mJ. The laser application of 1.8 mJ was insufficient, so the iridotomy opening was closed 7 days after the intervention, and we had to repeat LPI with a higher energy level. That happened to another patient treated with 2.8 mJ, so there were 2 patients in whom LPI had to be repeated after 7 days due to iridotomy closure caused by lower laser energy used.

To reduce iris thickness so that it can be perforated more easily, we applied Pilocarpine 2% (Pharmacy Zaječar, Serbia) and tetracaine hydrochloride 0.5% (Pharmacy Zaječar, Serbia) as the local anesthetic agent. Then, the Abraham lens (66-dioptre, 10 mm in diameter) was located with suitable hydroxypropyl-methylcellulose 2% gel (Galena, Belgrade). The site of iridotomy was chosen where the iris appeared thinnest, at a non-perpendicular angle directed toward the peripheral retina, to avoid possible macular laser coagulation.

The inclusion criteria were patients aged 40–79 years diagnosed with APAC with the pupillary block in one eye, ocular and periocular pain, nausea, vomiting, blurred vision with a halo effect surrounding a light source, IOP > 30 mmHg, corneal edema, ciliary injection, a medium-wide unreactive pupil, shallow anterior chamber leading to a forward shift of the peripheral iris, gonioscopically confirmed iridotrabecular contact in ≥ 3 quadrants, without glaucomatous change of the optic nerve head. The exclusion criteria were patients with APAC with GON, chronic angular glaucoma, secondary angle-closure glaucoma, previous intraocular surgery, corneal disease, macular degeneration, diabetic retinopathy, uveitis, patients on long-term anti-inflammatory therapy, and patients with cataract. All patients underwent a detailed ophthalmological examination – determination of the best-corrected visual acuity (VA), biomicroscopic examination, measuring IOP with the Goldmann applanation tonometer Digital Vision (Italy), fundus biomicroscopic examination with indirect ophthalmoscopy and +90 D lens, gonioscopy using a Goldmann 3 mirror lens, and automated static perimetry using the Humphrey Visual Field Analyzer, program 30–2 threshold. In this research, we presented changes in the angle width, IOP values, and Cup/Disc ratio during the follow-up monitoring of both eyes.

IOP values are easily measurable using the Goldmann applanation tonometer (GAT), mounted on a biomicroscope. IOP results are expressed in mmHg with the range from 10 to 21 mmHg in healthy individuals. IOP measurements were taken after 7 days, 1 month, and then every 3 months during the follow-up period.

To perceive the anatomy of the angle, we performed a gonioscopy using a biomicroscope with the Goldmann contact lens. The entire chamber angle was examined by 360-degree rotation, and the contact lens was placed on the corneal surface with the 1% methylcellulose solution. Gonioscopic angle width was graded in five categories from 0 mean closed to 4 mean wide open based on the Shaffer grading system.

The Vertical Cup/Disc value (Ver C/D rat) is useful in detecting glaucomatous damage. We determined it by examining the fundus by indirect ophthalmoscopy and the biomicroscope with a +90 D lens. The Ver C/D rat difference between both eyes and Ver C/D rat > 0.65 indicated glaucomatous damage. The closer the values are to 1.0, the more severe the damage.

We performed automated static perimetry using the threshold 30-2 test on the Humphrey Visual Field Analyzer based on the mean defect (MD) indices and Glaucoma Hemifield Test (GHT) results outside normal limits. The visual half-field test evaluated by Humphrey perimetry was used to compare groups of corresponding points above and below the horizontal meridian and showed visual field results denoted as "within normal limits", "outside normal limits", and "threshold values". When the values were "outside normal limits", it was a sign of disease progression.

Statistical data processing was carried out with the SPSS program, version 20.0. The results of $p < 0.05$ were considered statistically significant. Wilcoxon signed-rank test, contingency-table test, *t*-test for independent samples, and multivariate regression analysis were employed in the data processing.

Results

The research included 40 patients, 28 females and 12 males, with a mean age of 65.8 ± 11.8 years. At the time of contacting a doctor, based on the Shaffer grading system, the width of the angle in the APAC-affected eye was 0.15 ± 0.36 and 1.2 ± 0.41 in the fellow eye, and after 12 months, it was 0.85 ± 0.36 in the affected eye and 1.9 ± 0.36 in the fellow eye (Z = -5.91, $p < 0.01$) (Table 1).

The rates of MD progression and GHT results outside normal limits were found in 15 APAC-affected eyes (37.5%) of 9 females and 6 males. Disease progression was similar in both sexes ($\chi^2 = 0.51$, df = 1, $p > 0.05$), and they were all patients with moderate glaucoma defects MD < 12 dB. From the moment of detection until the end of the follow-up period, the average MD values were at the level of moderate glaucomatous defects. GHT results outside normal limits were not found in any healthy fellow eyes until the end of the follow-up period covering the research.

In 3 patients, there was a rise in IOP > 21 mmHg 7 days after LPI; after 6 months, IOP rose in other 4 patients, and after 12 months of follow-up, it rose in 7 additional patients, thus additional drug therapy was introduced, and in 3 of these patients, TTR was performed. There was no occurrence of cataracts during the follow-up period.

IOP was statistically significantly higher in patients with disease progression from the beginning, before the start of treatment, measuring 60.1 ± 4.37 mmHg compared to 53.6 ± 3.73 mmHg in patients without progression (t = -5.03, df = 38, $p < 0.01$) and over the time of monitoring. After 7 days, it was 20.67 ± 3.89 mmHg in the eye with progression compared to 17.52 ± 1.9 mmHg in the eye without progression (t = -2.93, df = 18.1, $p < 0.05$); after one month, the value was 19.87 ± 3.2mmHg in the eye with progression compared to 17.12 ± 2.26 in the eye without progression (t = -3.18, df = 38, $p < 0.05$); after 3 months, it was 21.73 ± 3.45 mmHg in the eye with progression compared to 16.88 ± 1.83 mmHg in the eye without progression (t = -5.04, df = 38, $p < 0.01$); after 6 months, the value was 22.53 ± 2.75 mmHg in the eye with progression compared to 16.08 ± 1.5 mmHg in the eye without progression (t = -8.38, df = 19.1, $p < 0.01$); after 9 months the value was 22.53 ± 2.59 mmHg in the eye with progression compared to 15.84 ± 1.57 mmHg in the eye without progression (t = -10.21, df = 38, $p < 0.01$); after 12 months the value measured was 23.4 ± 2.53 mmHg in the eye with progression compared to 14.92 ± 1.22 mmHg in the eye without progression (t = -14.29, df = 38, $p < 0.01$) (Table 2).

After 3 months from the beginning of treatment, the Ver C/D rat was significantly higher in the eye with disease progression (t = -4, df = 14, $p < 0.05$). It increased over time, and it showed a statistically highly significant difference in relation to the eye without disease progression after 6 months (t = -8.26, df = 14, $p < 0.01$), after 9 months (t = -14.7, df = 14, $p < 0.01$), and after 12 months of treatment (t = -19, df = 14, $p < 0.01$) (Table 3).

The largest number of patients, half of them (n = 20, 50%), contacted doctors within 12 hrs of the onset of symptoms, while the smallest number of patients (n = 3, 7.5%) consulted doctors between 12 and 24 hrs. Between 24 and 72 hrs of the onset of symptoms, 11 people contacted doc-

Table 1

Changes in angle width in the acute primary angle closure affected and fellow eyes

Period	Affected eye n = 40	Fellow eye n = 40	*p*-value
Before treatment	0.15 ± 0.36	1.2 ± 0.41	< 0.01
After 1 month	0.53 ± 0.36	1.51 ± 0.34	< 0.01
After 4 months	0.83 ± 0.39	1.88 ± 0.34	< 0.01
After 8 months	0.85 ± 0.36	1.9 ± 0.36	< 0.01
After 12 months	0.85 ± 0.36	1.9 ± 0.36	< 0.01

All values are expressed as mean ± standard deviation; Paired sample *t*-test, Wilcoxon rank test.

Table 2

Changes in intraocular pressure (IOP) in the eyes without (A) and with (B) disease progression

Period	IOP (mmHg)		*p*-value
	A (n = 25)	B (n = 15)	
Before treatment	53.6 ± 3.73	60.1 ± 4.37	< 0.01
After 7 days	17.52 ± 1.9	20.67 ± 3.89	< 0.05
After 1 month	17.12 ± 2.26	19.87 ± 3.2	< 0.05
After 3 months	16.88 ± 1.83	21.73 ± 3.45	< 0.01
After 6 months	16.08 ± 1.5	22.53 ± 2.75	< 0.01
After 9 months	15.84 ± 1.57	22.53 ± 2.59	< 0.01
After 12 months	14.92 ± 1.22	23.4 ± 2.53	< 0.01

All values are expressed as mean ± standard deviation; Independent samples *t*-test.

Table 3

Changes in the excavation of the optic nerve papilla in the eyes without (A) and with (B) disease progression

Period	Vertical Cup/Disc ratio		*p*-value
	A (n = 25)	B (n = 15)	
After 3 months	0.4 ± 0.1	0.45 ± 0.05	< 0.05
After 6 months	0.4 ± 0.1	0.53 ± 0.06	< 0.01
After 9 months	0.4 ± 0.1	0.57 ± 0.05	< 0.01
After 12 months	0.4 ± 0.1	0.65 ± 0.06	< 0.01

Independent samples *t*-test; All values are expressed as mean ± standard deviation.

tors (n = 11, 27.5%), and 6 of them waited for more than 72 hrs (n = 6, 15%), of which 3 patients (n = 3, 7.5%) underwent TTR. Multivariate regression analysis showed that the length of time before consulting a doctor is the most important factor in the progression of the disease, along with the IOP values in the second place.

There was neither increase above IOP normal values greater than 8 mmHg within 7 days after LPI nor the occurrence of later cataracts and other more serious complications during the follow-up period.

Discussion

APAC is one of the most urgent conditions in ophthalmology. Our research topic is particularly important in clinical work because it could save a lot of valuable time that is lost through diagnosing procedures. The research emphasizes the need for the reduction of high IOP values as soon as possible so that the cornea is transparent for timely LPI. Thus, well-maintained IOP values eliminate pupillary block and prevent APAC recurrence in the same eye as well as prophylactic LPI to prevent the occurrence of APAC in the fellow eye. The importance of timely care in terms of LPI performed in both eyes enables the prevention of disease progression to PACG. If LPI is not performed timely, irreversible vision loss occurs as a result, so it should be done as soon as possible, as the cornea is sufficiently clear because PACG is one of the leading causes of bilateral blindness in the world.

Many studies evaluate the effects of LPI in patients with APAC. Some studies show its effect on the angle width in these patients, such as the study by Lim et al. [17], where the average angle width was 0.7 before and 1.1 after LPI. The values of 0.25 before and 1.22 after LPI were reported by Moghimi et al. [18] and 0.82 before and 0.95 after LPI by Ahmadi et al. [11]. In studies dealing with follow-up after LPI, a rise in IOP was reported in 21–47% of eyes within a window of 6 to 18 months according to Rao et al. [19]. In two retrospective studies, additional treatment after LPI was necessary for 56% of eyes after 50 months as reported by Rao et al. [19] and for 67% after 46 months in the study by Pandav et al. [20], primarily drug therapy, while glaucoma surgery was performed in only 0–13% of patients, as Rao et al. [19] reported. This additional treatment has been discussed in many studies, including cataract surgery [20–24]. Drug treatment alone is not sufficient in the cases of angular glaucoma. All patients must undergo LPI as soon as possible to remove the pupillary block. If IOP control cannot be maintained following LPI and drug treatment, additional surgical treatment such as lens extraction and/or TTR is required. TTR proved to be effective but has a higher risk for postoperative development of cataracts and the shallower anterior chambers. Lens extraction can deepen the anterior chamber and open the chamber angle, thus preventing angle closure and disease progression to PACG [25]. Post-LPI eyes appeared to have a 47% lower risk of developing an acute attack or PAGG [13]. Progression to PACG occurred in 28.5% of subjects with APAC, according to the study by He et al. [26], which resulted from higher IOP values before the treatment, as reported by Rao et al. [19]. Our research demonstrated the efficacy of LPI in most patients with APAC, while in 15 patients (37.5%) the disease progressed to PACG. Our results are similar to Lai et al. [27]

and show the disease progression to PACG in patients with higher IOP values at the beginning of the disease. In our research, there was neither increase above IOP normal values greater than 8 mmHg within 7 days after LPI [16] nor the later occurrence and surgery of cataracts, which is another therapeutic option for patients with APAC [28], or other more serious complications during the follow-up period. The progression of the disease was mostly influenced by the duration of symptoms before consulting a doctor. Therefore, the longer the symptoms lasted, the worse the prognosis would get. Thus, trepano-trabeculectomy was performed in 3 patients who saw the doctor 72 hrs after the onset of symptoms, which is shorter than in the study by Aung et al. [29], where 26.1% of patients had symptoms more than a week before seeing a doctor. The results in our research were similar to those in the study by Tan et al. [30], where the average duration of symptoms before presentation was 28.2 hrs, and 75% of patients consulted the doctor on the first day. Our retrospective interventional cohort study demonstrated the efficacy of LPI in patients with unilateral APAC in preventing progression to PACG, as well as the prophylactic role of LPI in the fellow eye. A study published by Singh and Rijal [9] showed the efficacy of LPI in 78% of eyes in which IOP was well maintained after LPI. The great importance of prophylactic treatment of LPI has also been pointed out in the studies by Weinreb and Moghimi [31] and Koh et al. [32].

Conclusion

LPI is of great importance in preventing the progression from APAC-affected eye to PACG and also the occurrence of APAC in the fellow eye. Its effectiveness is mostly affected by the duration of symptoms before visiting a doctor and the level of IOP before starting treatment. In addition to its efficacy, it proved to be safe since no serious complications followed the treatment throughout the period of monitoring.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Quigley HA, Broman AT.* The number of people with glaucoma worldwide in 2010 and 2020. Br J Ophthalmol 2006; 90(3): 262–7.
2. *Ohnell H, Bengtsson B, Heijl A.* Making a correct diagnosis of glaucoma: Data from EMGT. J Glaucoma 2019; 28(10): 859–64.
3. *Foster PJ, Johnson GJ.* Glaucoma in China: how big is the problem? Br J Ophthalmol 2001; 85(11): 1277–82.
4. *Foster PJ, Aung T, Nolan WP, Machin D, Baasanhu J, Khaw PT,* et al. Defining "occludable" angles in population surveys: drainage angle width, peripheral anterior synechiae, and glaucomatous optic neuropathy in east Asian people. Br J Ophthalmol 2004; 88(4): 486–90.
5. *Seah SK, Foster PJ, Chew PT, Jap A, Oen F, Fam HB,* et al. Incidence of acute primary angle-closure glaucoma in Singapore. An island-wide survey. Arch Ophthalmol 1997; 115(11): 1436–40.
6. *Wang W, Song H, Liu Z.* Computational Study on the Biomechanics of Pupil Block Phenomenon. Biomed Res Int 2019; 2019: 4820167.
7. *Gupta R, Kumar R, Chauhan L.* Anterior chamber morphology changes in eyes with narrow angles by Scheimpflug imaging: pilocarpine versus laser peripheral iridotomy. Int Ophthalmol 2021; 41(6): 2099–108.
8. *Lachkar Y, Bouassida W.* Drug-induced acute angle closure glaucoma. Curr Opin Ophthalmol 2007; 18(2): 129–33.
9. *Singh P, Rijal AP.* Effectivity of Nd Yag PI in treatment of acute primary angle closure glaucoma. Nepal Med Coll J 2014; 16(1): 45–9.
10. *Liao C, Zhang J, Jiang Y, Huang S, Aung T, Foster PJ,* et al. Long-term effect of YAG laser iridotomy on corneal endothelium in primary angle closure suspects: a 72-month randomised controlled study. Br J Ophthalmol 2021; 105(3): 348–53.
11. *Ahmadi M, Naderi Beni Z, Naderi Beni A, Kianersi F.* Efficacy of neodymium-doped yttrium aluminum garnet laser iridotomies in primary angle-closure diseases: superior peripheral iridotomy versus inferior peripheral iridotomy. Curr Med Res Opin 2017; 33(4): 687–92.
12. *Yan C, Han Y, Yu Y, Wang W, Lyu D, Tang Y,* et al. Effects of lens extraction versus laser peripheral iridotomy on anterior segment morphology in primary angle closure suspect. Graefes Arch Clin Exp Ophthalmol 2019; 257(7): 1473–80.
13. *Kumar H, Mansoori T, Warjri GB, Somarajan BI, Bandil S, Gupta V.* Lasers in glaucoma. Indian J Ophthalmol 2018; 66(11): 1539–53.
14. *Moghimi S, Bijani F, Chen R, Yasseri M, He MG, Lin SC,* et al. Anterior segment dimensions following laser iridotomy in acute primary angle closure and fellow eyes. Am J Ophthalmol 2018; 186: 59–68.
15. *Park HS, Kim JM, Shim SH, Kim HT, Bae JH, Choi CY,* et al. Diurnal intraocular pressure changes in eyes affected with acute primary angle closure and fellow eyes after laser peripheral iridotomy. Jpn J Ophthalmol 2015; 59(5): 318–24.
16. *Waisbourd M, Shafa A, Delvadia R, Sembhi H, Molineaux J, Henderer J,* et al. Bilateral Same-day Laser Peripheral Iridotomy in the Philadelphia Glaucoma Detection and Treatment Project. J Glaucoma 2016; 25(10): e821–5.
17. *Lim LS, Aung T, Husain R, Wu YJ, Gazzard G, Seah SK.* Acute primary angle closure: configuration of the drainage angle in the first year after laser peripheral iridotomy. Ophthalmology 2004; 111(8): 1470–4.
18. *Moghimi S, Chen R, Johari M, Bijani F, Mohammadi M, Khodabandeh A,* et al. Changes in Anterior Segment Morphology After Laser Peripheral Iridotomy in Acute Primary Angle Closure. Am J Ophthalmol 2016; 166: 133–40.
19. *Rao A, Rao HL, Kumar AU, Babu JG, Madhulata U, Arthi J,* et al. Outcomes of laser peripheral iridotomy in angle closure disease. Semin Ophthalmol 2013; 28(1): 4–8.
20. *Pandav SS, Kaushik S, Jain R, Bansal R, Gupta A.* Laser peripheral iridotomy across the spectrum of primary angle closure. Can J Ophthalmol 2007; 42(2): 233–7.
21. *Gupta B, Angmo D, Yadav S, Dada T, Gupta V, Sihota R.* Quantification of Iridotrabecular contact in primary angle-closure disease. J Glaucoma 2020; 29(8): 681–8.
22. *Pearce FC, Thomas R, Wong NJ, Walland MJ.* Long-term progression after laser peripheral iridotomy in Caucasian primary angle closure suspects. Clin Exp Ophthalmol 2018; 46(7): 828–30.
23. *Radhakrishnan S, Chen PP, Junk AK, Nouri-Mahdavi K, Chen TC.* Laser peripheral iridotomy in primary angle closure: a report

by the American Academy of Ophthalmology. Ophthalmology 2018; 125(7): 1110–20.
24. *Day AC, Cooper D, Burr J, Foster PJ, Friedman DS, Gazzard G,* et al. Clear lens extraction for the management of primary angle closure glaucoma: surgical technique and refractive outcomes in the EAGLE cohort. Br J Ophthalmol 2018; 102(12): 1658–62.
25. *Baig N, Kam KW, Tham CC*. Managing Primary Angle Closure Glaucoma - The Role of Lens Extraction in this Era. Open Ophthalmol J 2016; 10: 86–93.
26. *He M, Jiang Y, Huang S, Chang DS, Munoz B, Aung T,* et al. Laser peripheral iridotomy for the prevention of angle closure: a single-centre, randomized controlled trial. Lancet 2019; 393(10181): 1609–18.
27. *Lai J, Choy BN, Shum JW*. Management of primary angle-closure glaucoma. Asia Pac J Ophthalmol (Phila) 2016; 5(1): 59–62.
28. *Husain R, Do T, Lai J, Kitnarong N, Nongpiur ME, Perera SA,* et al. Efficacy of phacoemulsification alone vs phacoemulsification with goniosynechialysis in patients with primary angle-closure disease: a randomized clinical trial. JAMA Ophthalmol 2019; 137(10): 1107–13.
29. *Aung T, Ang LP, Chan SP, Chew PT*. Acute primary angle-closure: long-term intraocular pressure outcome in Asian eyes. Am J Ophthalmol 2001; 131(1): 7–12.
30. *Tan AM, Loon SC, Chew PT*. Outcomes following acute primary angle closure in an Asian population. Clin Exp Ophthalmol 2009; 37(5): 467–72.
31. *Weinreb RN, Moghimi S*. Prophylactic laser iridotomy in primary angle closure suspects. Lancet 2019; 393(10181): 1572–4.
32. *Koh V, Keshtkaran MR, Hernstadt D, Aquino MCD, Chew PT, Sng C.* Predicting the outcome of laser peripheral iridotomy for primary angle closure suspect eyes using anterior segment optical coherence tomography. Acta Ophthalmol 2019; 97(1): e57–e63.

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Comparative evaluation of toxicology and sociodemographic characteristics in homicide and suicide victims

Uporedna analiza socijalno-demografskih i toksikoloških karakteristika žrtava ubistava i počinioca samoubistava

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Abstract

Background/Aim. Suicide and homicide are crucial social problems, especially frequent among the population younger than 40 years. The aim of this study was to assess the influence of different sociodemographic factors and relevant psychoactive substances on the difference between homicide and suicide victims. **Methods.** A cross-sectional study analyzed autopsy reports of 714 suicide and 166 homicide cases autopsied in five years (2011–2016). Out of these, 666 suicide and 127 homicide cases met the inclusion criteria for this study. Blood-ethanol concentration was determined by headspace gas chromatography with flame ionization detection. Analysis of substances other than ethanol was accomplished by gas chromatography-mass spectrometry and liquid chromatography with tandem mass spectrometry. **Results.** There was a significant difference in age, level of education, and employment rate between suicide and homicide cases ($p < 0.05$). The distribution of suicide and homicide cases differed significantly on weekdays compared to weekends [odds ratio(OR) = 1.5; 95 % confidence interval (CI) = 1–2.3; $p < 0.05$]. The presence of a psychoactive substance remained a nonsignificant predictor of whether a person would become a homicide or suicide victim ($p > 0.05$). Homicide victims were more likely to have significantly higher blood alcohol concentration (0.2–0.3 g/dL) than suicide victims (OR = 2.2; 95 % CI = 1–5; $p < 0.05$). **Conclusion.** The age, level of education, employment status, and high blood alcohol concentration (0.2–0.3 g/dL) of the victim were significantly different between suicide and homicide cases.

Key words:
autopsy; alcohol drinking; chromatography; education; homicide; risk factors; sex factors; sociodemographic factors; suicide; substance related disorders.

Apstrakt

Uvod/Cilj. Samoubistva i ubistva su veoma važan socijalni problem, koji je posebno čest kod osoba mlađih od 40 godina. Cilj rada bio je da se proceni uticaj socijalno-demografskih faktora i psihoaktivnih supstanci na razliku među žrtvama ubistava i počinioca samoubistava. **Metode.** Studijom preseka obuhvaćeno je 714 slučajeva samoubistava i 166 slučajeva ubistava, obdukovanih u periodu od pet godina (2011–2016). Nakon selekcije, u studiju je uključeno 666 slučajeva samoubistava i 127 slučajeva ubistava. Prisutvo alkohola u krvi određivano je metodom gasne hromatografije sa detekcijom plamene jonizacije. Analiza drugih supstanci, osim etanola, rađena je metodom gasne hromatografije sa masenom spektrometrijom i tečnom hromatografijom tandem-masenom spektrometrijom. **Rezultati.** Utvrđena je statistički značajna razlika u životnom dobu, nivou obrazovanja i statusu zaposlenja među slučajevima ubistava i samoubistava ($p < 0,05$). Distribucija slučajeva samoubistava i ubistava značajno se razlikovala radnim danima u odnosu na vikende [*odds ratio* (OR) = 1,5; 95 % *confidence interval* (CI) = 1–2,3; $p < 0,05$]. Prisustvo psihoaktivnih supstanci nije bilo značajan pokazatelj da li će osoba postati žrtva ubistva ili će izvršiti samoubistvo ($p > 0,05$). Žrtve ubistava su češće imale značajno veću koncentraciju alkohola u krvi (0,2–0,3 g/dL) nego žrtve samoubistva (OR = 2,2; 95 % CI = 1–5; $p < 0,05$). **Zaključak.** Starost, nivo obrazovanja, status zaposlenja i visoka koncentracija alkohola u krvi (0,2–0,3 g/dL) značajno su se razlikovali među žrtvama samoubistava i ubistava.

Ključne reči:
autopsija; alkohol, pijenje; hromatografija; obrazovanje; ubistvo; faktori rizika; pol, faktor; socijalno-demografski faktori; samoubistvo; poremećaji izazvani supstancama.

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Introduction

Suicide and homicide as forms of violent death represent conscious and intentional destruction of one's own or others' life. The frequency of these forms constantly declined between 2000–2015 on the global level [1, 2]. Different factors are associated with variations in homicide and suicide mortality rates. In previous decades, the influence of many social, economic, geographic, and demographic factors on the frequency of appropriate violent death types was particularly studied [3–10]. Furthermore, essential determinants connected with violence are generally the consumption of alcohol, different narcotics, and some psychoactive drugs [11–15]. Namely, previous studies showed that more than half of suicide and homicide victims had positive toxicological findings on psychoactive substances in the blood [11, 12, 16, 17]. However, presented results vary in different world regions, and many other factors such as gender, age, cause of death, and methodological procedures on data collection could affect the distribution of the presence of substances in suicide and homicide victims [14, 15, 18, 19].

The number of death cases due to injuries, poisoning, and other external causes shows a decreasing trend in Serbia [20]. Although distribution data of different forms of violent death exist, a relatively small number of studies examined the association between certain sociodemographic factors and the incidence of homicide and suicide in our region [2, 7]. In addition, the connection between psychoactive substance usage and these violent forms of death was insufficiently analyzed. Until now, only the influence of alcohol consumption on homicide and suicide rates has been extensively studied [5, 21].

The aim of this study was to assess the influence of different sociodemographic factors and relevant psychoactive substances on the difference between homicide and suicide victims.

Methods

Case identification

This study was designed as a cross-sectional. All homicide and suicide cases autopsied at the Institute of Forensic Medicine "Milovan Milovanović" in Belgrade, Serbia from 1st January 2011 to 31st December 2016 were included. From a total of 880 autopsy cases, 127 homicide and 666 suicide cases fulfilled the selection criteria. Considering that the aim of our study was to examine the indirect influence of psychoactive substances on suicide and homicide frequency, all those cases in which the cause of death was deliberate or accidental drug overdose were excluded. All cases in which the victim died in a hospital after being treated for 24 hrs without undergoing toxicological analysis were also excluded. After the selection, 793 cases (127 homicides and 666 suicides) remained in our study. For this type of study, formal consent was not required.

Toxicological analysis

Toxicological analyses were performed in the Laboratory of Toxicology of the Institute of Forensic Medicine "Milovan Milovanović". The presence and concentration of ethanol were determined in all cases; however, not all suicide and homicide victims were examined for the presence of other psychoactive substances. Hence, the influence of psychoactive drugs besides ethanol on homicide and suicide rates and their other features was analyzed only in cases with complete toxicological findings (47 homicide and 129 suicide cases).

Blood-alcohol concentration (BAC) was determined by head-space gas chromatography with flame-ionization detection (HS-GC-FID). The limit of ethanol detection was set at 0.0001 g/dL, and the limit of quantification was 0.0003 g/dL. In this study, the cut-off concentration was 0.03 g/dL to report positive alcohol results because lower values can be the result of endogenous *post-mortem* alcohol production [22].

Analysis of other psychoactive substances was performed by gas chromatography with mass spectrometry (GC-MS) and liquid chromatography with tandem mass spectrometry (LC-MS/MS).

Case categorization

All included cases of homicide and suicide were divided into categories according to the victim's gender, age, marital status, level of education, employment status, day of death, suicidal or homicidal method, and toxicological analysis findings. To simplify our analysis, we defined age groups as < 40, 40–65, and > 65 years of age. According to the education level, subjects were divided into two categories, those with a high level of education (if the victim had a university degree) and those with lower levels of education (finished elementary and/or high school). The day of death was categorized into a weekend (Friday, Saturday, and Sunday) or a workday, accordingly. Psychoactive substances were categorized as alcohol (ethanol), methadone and opioids (heroin, 6-monoacetylmorphine, codeine, morphine, and/or tramadol), cannabinoids (D-9-tetrahydrocannabinol – THC and/or THC acid), psychostimulants (cocaine, benzoylecgonine, and/or 3,4 methylenedioxymethamphetamine – DMA), and psychoactive drugs (benzodiazepines, antidepressants, antipsychotics, and/or anticonvulsants). Heavy alcohol intoxication was defined as the presence of BAC of 0.2–0.3 g/dL.

Statistical analysis

Statistical analysis was conducted using the commercial statistical package SPSS (Statistical Package for the Social Sciences software – IBM Statistics) version 20.0. The evaluation of differences between variables was performed by Student's *t*-test and Mann-Whitney *U* test depending on the normality of data distribution. In order to define the influence of psychoactive substances and concordant social demographic factors on homicide or suicide risk, the odds ratio (OR) and 95% confidence interval (CI) were estimated using logistic regression. A *p*-value below 0.05 was considered significant, and below 0.01 was considered highly significant.

Results

The victim's demographic and forensic characteristics are shown in Table 1. There was no statistically significant difference in gender distribution between suicide and homicide cases ($p = 0.062$). According to the mean age between homicide and suicide victims, the difference was statistically significant ($p < 0.001$).

The distribution of homicide and suicide victims according to age groups is shown in Figure 1. Persons aged under 40 years were significantly more frequent among homicide victims compared to suicide victims (OR = 3.8; 95% CI = 2.5–5.6; $p < 0.01$). Marital status in homicide cases was not significantly different from suicide cases. There were significantly more victims with a higher level of education in the suicide group (15.9%) compared to the homicide group (7.8%). Better-educated persons had a lower risk of being killed than committing suicide (Table 1).

Among homicide cases, more victims were employed compared to the suicide cases. The most common homicidal method was a gunshot (55.6%), whereas, in the suicide group, it was hanging (42.3%) (Table 1). The distribution of cases depending on the day of death is shown in Figure 2. Those who died on workdays were more often victims of homicide in relation to those killed on weekends (OR = 1.5; 95% CI = 1–2.3; $p = 0.049$).

Table 1

Demographic and forensic characteristics of homicide and suicide cases

Variable	Homicide	Suicide	OR (95% CI)	*p*
Age	45.2 ± 17.34	57.3 ± 17.4		< 0.001
Gender				
male	85 (66.9)	499 (74.9)	1.0	
female	42 (33.1)	167 (25.1)	1.5 (1–2.2)	0.062
Married				
no	68 (56.2)	370 (57.1)	1.0	
yes	53 (43.8)	278 (42.9)	1.0 (0.7–1.5)	0.854
Education (higher level)				
no	106 (92.2)	499 (84.1)	1.0	
yes	9 (7.8)	94 (15.9)	0.4 (0.2–0.9)	0.029
Employed				
no	81 (67.5)	489 (76.8)	1.0	
yes	39 (32.5)	148 (23.2)	1.6 (1–2.4)	0.032
Method				
gunshot	70 (55.6)	170 (25.5)		
cuts/stabbing	29 (23)	29 (4.4)		
fall from height	0	122 (18.3)		
hanging	0	282 (42.3)		
blunt force injury	18 (14.3)	0		
drowning	0	33 (5)		
poisoning	0	9 (1.4)		
vehicle injury	0	10 (1.5)		
strangulation/asphyxia	7 (5.6)	2 (0.3)		
other	2 (1.6)	9 (1.4)		

OR – Odds ratio; CI – confidence interval.
All values are expressed as numbers (percentages) except for the age, which is expressed as mean ± standard deviation.

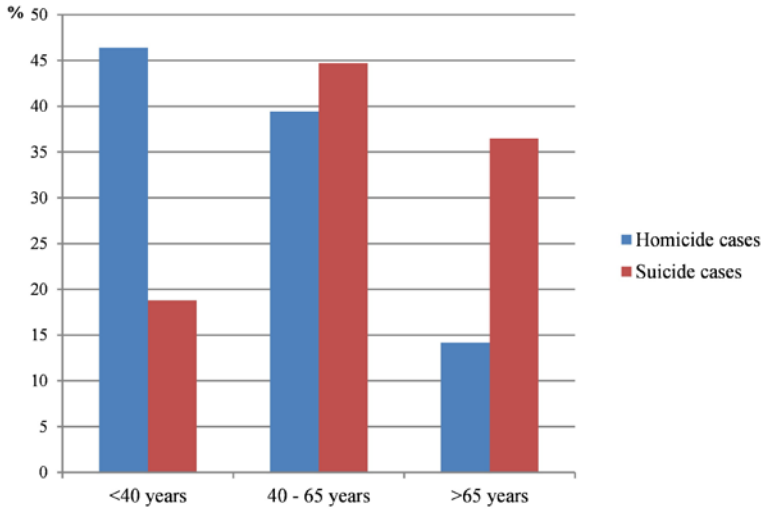

Fig. 1 – Distribution of suicide and homicide cases according to age groups.

The relationship between toxicological findings and the distribution of homicide and suicide cases is presented in Table 2. There were no statistically significant differences in the presence of opioids and methadone, psychostimulants, and psychoactive drugs between the group of homicide and suicide cases. As cannabis was not detected in any of the suicide cases, its influence could not be examined.

Alcohol (ethanol) was present in 15.5% of all cases (18.1% of homicide and 15% of suicide cases); however, its presence did not affect the distribution of victims owing to appropriate forms of violent death. The median blood-ethanol concentration of homicide victims was 0.19 g/dL (ratio 0.04–0.48 g/dL), while in the population of suicide victims, it was 0.14 g/dL (ratio 0.04–0.51 g/dL), and the difference was not significant (U = 909.000; p = 0.12). Nevertheless, the blood-ethanol concentration corresponding to heavy alcohol intoxication (0.2–0.3 g/dL) was significantly more frequent in homicide victims (7.1% to 3.3% OR = 2.2; 95% CI = 1–5; p = 0.049).

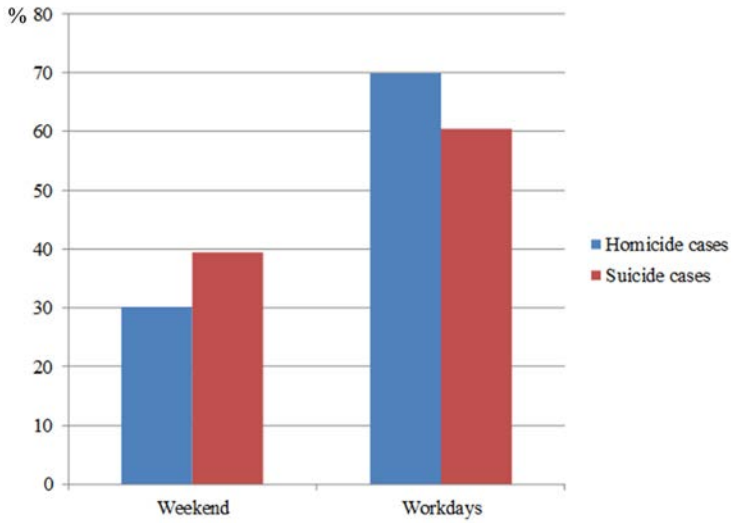

Fig. 2 − Distribution of suicide and homicide cases according to the day of death.

Table 2

Toxicology results of homicide and suicide cases

Variable	Homicide	Suicide	OR (95% CI)	*p*
Alcohol				
no	104 (81.9)	566 (85)		
yes	23 (18.1)	100 (15)	1.3 (0.8–2.1)	0.38
Blood-ethanol concentration (g/dL)	0.19 (0.04–0.48)	0.14 (0.04–0.51)		0.12
High level of alcohol intoxication (0.2–0.3 g/dL)				
no	118 (92.9)	644 (96.7)		
yes	9 (7.1)	22 (3.3)	2.2 (1–5)	0.04
Opioids and methadone				
no	42 (89.4)	111 (86)		
yes	5 (10.6)	18 (14)	0.7 (0.3–2.1)	0.56
Cannabis				
no	44 (93.6)	129 (100)		
yes	3 (6.4)	0		
Psychostimulants				
no	45 (95.7)	128 (98.5)		
yes	2 (4.3)	2 (1.5)	2.8 (0.4–20.8)	0.30
Psychoactive drugs				
no	41 (87.2)	96 (74.4)		
yes	6 (12.8)	33 (25.6)	0.4 (0.2–1.1)	0.07
Illicit substances				
no	39 (83)	113 (87.6)		
yes	8 (17)	16 (12.4)	1.4 (0.6–3.6)	0.43

OR – Odds ratio; CI – confidence interval.
All values are expressed as numbers (percentages) except for the blood-ethanol concentration, which is expressed as median and range.

Discussion

In particular world regions such as India, the USA, and Japan, the incidence of homicide and suicide is stagnant or rising [2, 23, 24]. On the other hand, in some countries, these forms of lethal violence are considered the most common causes of death in the population under 40 years of age [25]. The effect of these forms of violent death on overall mortality rates will likely become more notable in the future following numerous societal and technological changes worldwide. Sociodemographic characteristics of the population and the abuse of psychoactive substances probably play an important role in the cases of suicide and homicide, but their influence is still insufficiently estimated.

Our study examined the influence of different sociodemographic factors and relevant psychoactive substances on the difference between homicide and suicide victims. It was shown that certain sociodemographic characteristics (e.g., gender, educational level, marital status, etc.) could increase or decrease a person's risk of becoming a victim of homicide or suicide. Nevertheless, it was shown that there was no significant difference between homicide and suicide cases in terms of the usage and type of abused psychoactive substances.

In our study, there was a significant age difference between the homicide and suicide populations. Younger persons were more prevalent in the group of murder victims, while older more often committed suicide. These results could, to some extent, explain the differences in homicide and suicide rates between younger and older persons. Our results are in concordance with numerous studies that showed that most of the population of suicide victims are persons older than 65 years, whereas victims of murder were most often persons under 40 years of age [5, 18, 25–27]. The older population is prone to developing different organic diseases connected with certain psychiatric disorders. Moreover, the cumulative effect of stress increases with age, and it can negatively affect mental health and contribute to suicidal behavior [27]. On the contrary, the population younger than 40 years is more exposed to various circumstances associated with interpersonal violence [4, 28].

The previous studies also showed male predominance in homicide and suicide cases, and our study showed that the distribution of males and females did not differ significantly between homicide and suicide victims, which is consistent with the results of Molina and Hargrove [16]. In contrast, Darke et al. [11] found that males are more prone to suicide, three times as often as women. This difference could be explained by the fact that in some parts of the world, females primarily choose methods to commit suicide that do not result in immediate death. That could lead to an underestimate of the number of suicide cases, even though there is an equal or higher prevalence of suicide attempts in females compared to males [7, 29].

Like in other studies [9, 29], our results showed no significant association between marital status and type of violent death. Obtained results also pointed out that suicide and homicide cases did not differ significantly in terms of the victim's marital status. However, our results indicated that the level of education and employment status could have a specific influence on homicide and suicide rates. Highly educated and unemployed persons had a lower risk of becoming murder victims than committing suicide. In contrast, the employed had a greater risk of being killed. Our results are in concordance with the data of Bando and Lester [9] and Milner et al. [30]. It is considered that a population of highly educated individuals is more likely to avoid engaging in dangerous behavior. Likewise, these people are less exposed to a social environment where interpersonal violence plays an important role in dealing with misery and unhappiness [9]. Highly educated persons, in most cases, have a better quality of life and thus often have no clear external source to blame for their misery. That could lead to depression and specific autoaggressive behavior. On the contrary, persons with a lower quality of life mainly represent the poorly educated people who solve their conflicts with violence that could result in murder [31]. Prolonged unemployment is usually followed by a sense of underachievement and self-deprecation that could result in vulnerability to suicide [30].

In the presented report, more murders were observed on workdays rather than suicides. The possible explanation could be careful planning of the murder as well as knowledge of the daily routine of the victim. As opposed to these, suicides were more frequent on weekends. However, fewer studies have analyzed the association between the day of death and suicide and homicide rates. Kattimani et al. [32] reported in their study that Sunday is the day with the highest number of suicide attempts.

In agreement with several previous studies [11, 12, 16, 17], more than half of included homicide and suicide victims were positive for a psychoactive substance. These drugs have direct psychopharmacological effects on the user, making them more prone to endanger their own or others' health. The consumer is also often forced to use violence (through robbery or burglary) to obtain money for drugs. Finally, illicit drugs cause systemic violence as a result of activities on illegal drug markets, where conflicts are regularly solved using violent behavior [12]. Regardless of the obtained results, the influence of psychoactive substance uses on violence, in general, cannot be ignored.

Ethanol was detected in 15.5% of all analyzed suicide and homicide cases. Results of our study showed that the presence of ethanol in the blood did not differ significantly between suicide and homicide victims. This finding was in accordance with results obtained by Darke et al. [11]. Despite this finding, the fact that 4.7% of the population in Serbia consumes alcohol daily [20] emphasizes the importance of alcohol in the expression of lethal violence. Darke et al. [11] also found that the concentration of ethanol was significantly higher in murdered persons than that among suicide victims; however, our study did not show a similar difference between homicide and suicide cases. In the present study, a significantly higher number of murder victims were in a state of heavy alcohol intoxication (0.2–0.3 g/dL) in comparison to suicide victims. That can imply that high blood-ethanol concentrations have a particular influence on differences be-

tween homicide and suicide rates. Additionally, a possible explanation for this finding could be that victims with heavy alcohol intoxication are more vulnerable and are less competent to perform complicated forms of suicide such as hanging.

In our study, there was no significant difference between homicide and suicide cases in terms of the presence of opioids, psychostimulants, and psychoactive drugs. On the contrary, other studies [11, 16] showed that the presence of opioids and psychostimulants was more prevalent among the murdered than the suicide victims, while certain psychoactive drugs were more often detected in the victims of suicide. On the one hand, the insufficient number of complete toxicological analyses directly affected the sample size of our study. On the other hand, unjustified and uncontrolled usage of certain psychotropic drugs in the general population could explain our results.

Conclusion

The results of our study pointed out that some sociodemographic characteristics, primarily age, level of education, and employment status, could affect the differences between the victims of homicide and suicide. Additionally, murders were more prevalent on workdays, while suicides occurred more frequently on weekends. The presence of a psychoactive substance was not associated with differences between homicide and suicide rates. Heavy alcohol intoxication could increase the chances of becoming a murder victim in comparison to committing suicide.

Considering that some of the toxicological findings could be predictors of homicide and suicide, it should be advised that the complete toxicological analysis be performed not only in the herein discussed but also in all other forms of violent deaths in the future.

REFERENCES

1. World health statistics 2017: monitoring health for the SDGs, Sustainable Development Goals. Geneva: World Health Organization; 2017.
2. *Ilic M, Ilic I.* Suicide in Serbia. J Affect Disord 2016; 193: 187–93.
3. *Fountoulakis KN, Chatzikosta I, Pastiadis K, Zanis P, Kawohl W, Kerkhof AJ,* et al. Relationship of suicide rates with climate and economic variables in Europe during 2000–2012. Ann Gen Psychiatry 2016; 15: 19.
4. *Mundia L, Matzin R, Mahalle S, Hamid MH, Osman RS.* Contributions of sociodemographic factors to criminal behaviour. Psychol Res Behav Manag 2016; 9: 147–56.
5. *Rancic N, Erceg M, Radojevic N, Savic S.* Medicolegal characteristics of firearm homicides in Belgrade, Serbia: before, during, and after the war in the former Yugoslavia. J Forensic Sci 2013; 58(6): 1549–53.
6. *Kovacević D, Zarković-Palijan T, Radeljak S, Marinović D, Hero ED, Golub TL.* Forensic aspects in domestic homicide. Coll Antropol 2010; 34(Suppl 2): 29–37.
7. *Dedic G.* Gender differences in suicide in Serbia within the period 2006-2010. Vojnosanit Pregl 2014; 71(3): 265–70.
8. *Santric Milicevic M, Bjegovic V, Terzic Z, Vukovic D, Kocev N, Marinkovic J,* et al. Serbia within the European context: An analysis of premature mortality. Popul Health Metr 2009; 7: 12.
9. *Bando DH, Lester D.* An ecological study on suicide and homicide in Brazil. Cien Saude Colet 2014; 19(4): 1179–89.
10. *Kalediene R, Starkuviene S, Petrauskiene J.* Seasonal patterns of suicides over the period of socio-economic transition in Lithuania. BMC Public Health 2006; 6: 40.
11. *Darke S, Duflou J, Torok M.* Drugs and violent death: comparative toxicology of homicide and non-substance toxicity suicide victims. Addiction 2009; 104(6): 1000–5.
12. *Hedlund J, Ahlner J, Kristiansson M, Sturup J.* A population-based study on toxicological findings in Swedish homicide victims and offenders from 2007 to 2009. Forensic Sci Int 2014; 244: 25–9.
13. *Kuhns JB, Maguire ER.* Drug and alcohol use by homicide victims in Trinidad and Tobago, 2001-2007. Forensic Sci Med Pathol 2012; 8(3): 243–51.
14. *Delaveris GJ, Teige B, Rogde S.* Non-natural manners of death among users of illicit drugs: Substance findings. Forensic Sci Int 2014; 238: 16–21.
15. *Kuhns JB, Wilson DB, Clodfelter TA, Maguire ER, Ainsworth SA.* A meta-analysis of alcohol toxicology study findings among homicide victims. Addiction 2010; 106(1): 62–72.
16. *Molina DK, Hargrove VM.* Can intoxication status be used as a prediction tool for manner of death: A comparison of the intoxication status in violent suicides and homicides. Am J Forensic Med Pathol 2017; 38(1): 69–73.
17. *Darke S.* The toxicology of homicide offenders and victims: a review. Drug Alcohol Rev 2010; 29(2): 202–15.
18. *Cros J, Alvarez JC, Sbidian E, Charlier P, Lorin de la Grandmaison G.* Homicidal deaths in the Western suburbs of Paris: a 15-year-study. Am J Forensic Med Pathol 2012; 33: 404–9.
19. *Skibin L, Bilban M, Balazic J.* Harmful alcohol use of those who died a violent death (the extended region of Ljubljana 1995–1999). Forensic Sci Int 2005; 147(Suppl): S49–52.
20. Institute of Public Health of Serbia. Health statistical yearbook of the Republic of Serbia 2016; Belgrade: Institute of Public Health of Serbia "Dr. Milan. Jovanovic Batut"; 2017.
21. *Cvetković D, Živković V, Lukić V, Nikolić S.* Unnatural and Violent Death in Cases with High Blood Alcohol Concentration-Autopsy Study. J Forensic Sci 2017; 62(6): 1506–11.
22. *Kugelberg FC, Jones AW.* Interpreting results of ethanol analysis in postmortem specimens: a review of the literature. Forensic Sci Int 2007; 165(1): 10–29.
23. *Rockett IR, Regier MD, Kapusta ND, Coben JH, Miller TR, Hanzlick RL,* et al. Leading Causes of Unintentional and Intentional Injury Mortality: United States, 2000–2009. Am J Public Health 2012; 102(11): e84–e92.
24. *Reddy MS.* Suicide incidence and epidemiology. Indian J Psychol Med 2010; 32(2): 77–82.
25. *Richardson EG, Hemenway D.* Homicide, suicide, and unintentional firearm fatality: comparing the United States with other high-income countries, 2003. J Trauma Acute Care Surg 2011; 70(1): 238–43.
26. *Fässberg MM, van Orden KA, Duberstein P, Erlangsen A, Lapierre S, Bodner E,* et al. A Systematic review of social factors and suicidal behavior in older adulthood. Int J Environ Res Public Health 2012; 9(3): 722–45.
27. *Draper BM.* Suicidal behaviour and suicide prevention in later life. Maturitas 2014; 79(2): 179–83.
28. *Lachaud J, Donnelly PD, Henry D, Kornas K, Calzavara A, Bornbaum C,* et al. A population-based study of homicide deaths in Ontario, Canada using linked death records. Int J Equity Health 2017; 16(1): 133.
29. *Ambade VN, Godbole HV, Kukde HG.* Suicidal and homicidal deaths: a comparative and circumstantial approach. J Forensic Leg Med 2007; 14: 253–60.

30. *Milner A, Page A, LaMontagne AD*. Long-term unemployment and suicide: a systematic review and meta-analysis. PLoS One 2013; 8(1): e51333.
31. *Fountoulakis KN, Gonda X*. Differential correlation of suicide and homicide rates according to geographical areas: A study with population-level data. Psychiatry Res 2017; 249: 167–71.
32. *Kattimani S, Penchilaiya V, Sarkar S, Muthukrishnan V*. Temporal variations in suicide attempt rates: A hospital-based study from India. J Family Med Prim Care 2016; 5: 357–61.

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Effectiveness of submucosal, oral, and intramuscular routes of dexamethasone administration in trismus, swelling, and pain reduction after the third lower molar surgery

Efikasnost submukozne, oralne i intramuskularne primene deksametazona u redukciji trizmusa, otoka i bola nakon hirurgije donjih trećih molara

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Abstract

Background/Aim. Surgical extraction of impacted lower third molars is inevitably followed by the postoperative occurrence of trismus, swelling, and pain sensations to some degree. Corticosteroids (dexamethasone in particular) are commonly used drugs in the prevention of these complications. The aim of this study was to determine the effectiveness of dexamethasone in the prevention of postoperative complications, edema, trismus, and pain after the surgical extraction of impacted lower third molars, depending on the method of its administration. **Methods.** This prospective study involved 30 healthy patients, aged 18 years and above, of both sexes, with fully impacted lower third molar – class I or II and position B or C, according to Pell and Gregory classification system and vertical position according to Winter classification. All patients were divided randomly into three groups depending on the way of dexamethasone administration: oral – dexamethasone administered in the form of oral tablets in a dose of 4 mg one hour before the surgery; submucosal – dexamethasone solution administered submucosally in a dose of 4 mg in the area of the buccal sulcus, after the inferior alveolar nerve block anesthesia and additional anesthesia for the buccal nerve; intramuscular – dexamethasone solution administered intramuscularly in a dose of 4mg into the area of the deltoid muscle, right before the intervention. Preoperatively and at every follow-up (on the first, second, and seventh day postoperatively), interincisal distance, the degree of edema, and the level of pain with the use of a visual analog scale (VAS) were measured. On the seventh postoperative day, the total number of analgesics taken by the patients was recorded. **Results.** In the postoperative period, there was no statistically significant difference between the examined groups in terms of effectiveness in swelling, trismus, and pain reduction ($p > 0.05$). **Conclusion.** There is no significant difference in dexamethasone effectiveness in postoperative trismus, swelling, and pain reduction after the third lower molar surgery, regarding the route of administration – oral, intramuscular, or local submucosal.

Key words:
dexamethasone; drug administration routes; molar, third; oral surgical procedures; trismus.

Apstrakt

Uvod/Cilj. Hirurška ekstrakcija impaktiranih donjih trećih molara je, u izvesnom stepenu, neizbežno praćena postoperativnom pojavom trizmusa, otoka i osećaja bola. Kortikosteroidi (naročito deksametazon) su lekovi koji se najčešće koriste u prevenciji tih komplikacija. Cilj rada bio je da se utvrdi efikasnost deksametazona u prevenciji postoperativnih komplikacija, edema, trizmusa i bola, nakon hirurške ekstrakcije impaktiranih donjih trećih molara, u zavisnosti od načina njegove administracije. **Metode.** Prospektivnom studijom obuhvaćeno je 30 zdravih pacijenata, starijih od 18 godina, oba pola, sa potpuno impaktiranim donjim trećim molarom – klase I ili II i pozicije B ili C, prema klasifikaciji *Pell*-a i *Gregory*-ja, i vertikalne pozicije prema klasifikaciji *Winter*-a. Svi pacijenti su nasumično podeljeni u tri grupe u zavisnosti od načina primene deksametazona: oralno – deksametazon primenjen u obliku oralnih tableta, u dozi od 4 mg, sat vremena pre operacije; submukozno – rastvor deksametazona primenjen submukozno, u dozi od 4 mg, u predelu bukalnog sulkusa, nakon sprovodne anestezije za donji alveolarni nerv i

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dodatne anestezije za bukalni nerv; intramuskularno – rastvor deksametazona primenjen intramuskularno, u dozi od 4 mg, u predelu deltoidnog mišića, neposredno pre intervencije. Preoperativno i pri svakoj kontrolnoj poseti (prvog, drugog i sedmog dana postoperativno) određivani su interincizalno rastojanje, stepen edema i stepen bola primenom vizuelno analogne skale (VAS). Sedmog postoperativnog dana evidentiran je ukupan broj analgetika koje su pacijenti uzimali. **Rezultati**. U postoperativnom periodu nije bilo statistički značajne razlike između ispitivanih grupa u pogledu efikasnosti u smanjenju otoka, trizmusa i bola ($p > 0,05$). **Zaključak**. Nema značajne razlike u efikasnosti deksametazona u odnosu na način primene – oralno, intramuskularno ili lokalno submukozno, u redukciji postoperativnog trizmusa, otoka i bola nakon hirurškog lečenja impaktiranog donjeg trećeg molara.

Ključne reči:
deksametazon; lekovi, putevi primene; molar, treći; hirurgija, oralna, procedure; trizmus.

Introduction

Surgical extraction of impacted lower third molars is one of the most frequent procedures in oral surgery. Tissue trauma, made during the operation, causes a response in the form of hyperemia, vasodilation, increased vascular permeability, as well as granulocyte and monocyte migration [1, 2]. That is followed by the appearance of pain sensations, swelling, and trismus, which negatively impact a patient's quality of life in the early postoperative period [3]. Besides the physical methods (placement of a rubber drain or the use of photodynamic therapy), corticosteroids are commonly used in the treatment of these postoperative complications [4, 5].

Administration of corticosteroids in order to prevent postoperative complications after surgical extraction of the impacted lower third molars is a common and very effective pharmacological method [4–6]. There are two main classes of corticosteroids: mineralocorticoids and glucocorticoids. Due to their anti-inflammatory potential, glucocorticoids are used in oral surgery. Based on the duration of action and anti-inflammatory potency, this group of drugs can be classified into: short-acting, including hydrocortisone and cortisol, with a duration of action of up to 12 hrs and anti-inflammatory potential 1; medium-acting, which includes methylprednisolone, with a duration of action from 12 to 36 hrs and anti-inflammatory potential 4; long-acting, which includes dexamethasone and betamethasone, with a duration of action of over 36 hrs and anti-inflammatory potential 25 [7]. Glucocorticoids reduce inflammation in several ways. They inhibit the activity of the enzyme phospholipase A2, block the synthesis of prostaglandins and leukotrienes, which are considered mediators of inflammation, stabilize the cell membrane and thus reduce the release of inflammatory mediators, blood vascular permeability, and formation of bradykinin, which has a pronounced vasodilator effect [8].

Dexamethasone is one of the most frequently studied and most commonly used types of corticosteroids in oral and maxillofacial surgery today. Numerous studies indicate its positive effect on swelling, pain, and trismus reduction after the third molar surgery [6, 9–12]. In that sense, it can be administered *via* the local route – endoalveolar or submucosal administration, and *via* the systemic route – oral, intravenous, and intramuscular administration. There is still no consensus on the best route of its administration in order to prevent postoperative sequelae [9, 10].

The better effect of dexamethasone in postoperative complications prevention compared to other types of corticosteroids is well documented in literature [8, 13, 14]. Furthermore, the fact that the effect of dexamethasone can be observed for up to three postoperative days allows the use of this medication in only one dose for preventing postoperative trismus, pain, and swelling. Regarding the dosing regimen, the literature states a wide range of doses in which dexamethasone can be administered to prevent postoperative complications, but it can be said that its minimum effective dose is 4 mg. Several studies indicate that there is no statistically significant difference in the effect of dexamethasone among different dose usages [15–17]. The aim of this study was to compare the effectiveness of dexamethasone for the prevention of postoperative complications, edema, trismus, and pain after the surgical extraction of impacted lower third molars, depending on the method of its administration.

Methods

The protocol of this study was approved by the Ethics Committee of the Faculty of Medical Sciences in Priština/Kosovska Mitrovica with protocol No. 09-453, from March 03, 2021. The study was conducted as a prospective study, involving a total of 30 patients, aged 18 years and above, of both sexes, with fully impacted lower third molar – class I or II and position B or C, according to Pell and Gregory [18] classification system and vertical position according to Winter classification [19]. All patients were divided randomly into three groups depending on the way dexamethasone was administered: oral – dexamethasone administered in the form of oral tablets in a dose of 4 mg (Dexason® tab. 0.5 mg, Galenika, Serbia) one hour before the surgery (n = 10); submucosal – dexamethasone solution administered submucosally, in a dose of 4 mg (Dexason® amp 4 mg/mL, Galenika, Serbia), in the area of the buccal sulcus, at the site where the future flap will be formed, after the inferior alveolar nerve block anesthesia and additional anesthesia for the buccal nerve (n = 10); intramuscular – dexamethasone solution administered intramuscularly, in a dose of 4 mg (Dexason® amp 4 mg/mL, Galenika, Serbia), into the area of the deltoid muscle, right before the intervention (n = 10). Patients with systemic diseases, gastric ulcers, pregnant and lactating women, and people allergic to the drugs used in the study were not included. Furthermore, surgical procedures lasting longer than 60 min, as well as the occurrence of severe surgical

complications like infection or alveolar osteitis, were some of the reasons for exclusion from the study.

Preoperatively, the position of the impacted mandibular third molar was analyzed using an orthopantomogram according to Winter [19] classification and Pell and Gregory [18] classification. In addition, clinically, before the procedure, the distance between the cutting edges of the upper and lower incisors was measured, together with the parameters that would be used as a reference for determining the degree of postoperative edema. Surgical extractions were performed under local anesthesia – inferior alveolar nerve block with the additional plexus anesthesia for the *nervus buccalis* (Ubistesin forte®, 1:100,000, Ultradent, Germany), using a buccal triangular flap. Alveolotomy and, if necessary, separation of the crown and roots of the impacted teeth were performed. After the surgical extraction, patients were advised to apply ice packs for the first six hours after surgery and use the analgesic Paracetamol at a dose of 500 mg in combination with Caffeine 65 mg (Panadol extra® tab. 500 mg + 65 mg, GlaxoSmithKline, Republic of Ireland), as needed, up to a maximum of four tablets daily. Patients were asked to keep a record of the total number of analgesics they used until the seventh postoperative day. Patients were prescribed antibiotic therapy (Erythromycin® 0.5g – Hemofarm AD, Vršac, Serbia) every six hours for five days.

Postoperative follow-up was performed on the first, second, and seventh postoperative day in order to record the degree of edema, trismus, and pain.

Assessment of the degree of edema was measured according to the method of Schultze-Mosgau et al. [20], which involves measuring the distance between the tragus and the corner of the lips, tragus and pogonion, as well as the lateral angle of the eye and the mandible angulus. For this purpose, a silk thread was used to measure the distance between two points, and then those measurements were transferred to a millimeter ruler. The arithmetic mean values of these three variables were calculated for each patient. Obtained data were then compared with the measures obtained in the preoperative period.

The degree of trismus was determined by the distance between the incisal edges of the upper and lower central incisors on the maximal mouth opening, measured in millimeters with a ruler, and it was also compared to the preoperatively collected data.

The level of pain was determined on every follow-up visit, measured with a visual analog scale (VAS), graded in centimeters from 0 to 10, where 0 was the lowest notch marking the "absence of pain" while notch 10 marked "unbearable pain". The degree of pain was additionally evaluated by the total number of analgesic drugs consumed by the patient in the period of seven days.

According to the type of variables and the normality of the distribution, the data description is shown as n (%), mean ± standard deviation (SD), or median (min-max). Repeated measures analysis of variance (ANOVA) and linear mixed models (LMNMS) were used to model the correlation between the size of the swelling, trismus, and VAS pain scale over time as dependent variables in relation to the type of corticosteroid administration (submucosal, oral, intramuscular) (Table 1). The level of statistical significance was set at 0.05.

All data were processed using IBM SPSS Statistics 22 (SPSS Inc., Chicago, IL, USA) software package and R-3.6.3 software environment (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Swelling

Preoperatively, the arithmetic mean and SD of the swelling volume was 12.4 ± 1.8 cm in the submucosal group, 11.6 ± 0.7 cm in the oral group, while in the intramuscular group, it was 12.0 ± 1.5 cm, which is not a statistically significant difference ($p = 0.426$). The distribution of patients among groups was valid (Table 2).

Overall, there was a significant reduction in the size of the swelling in the examination period ($p < 0.001$) among all

Table 1

Total number of respondents and their distribution according to the method of administration

Parameter	Frequency	%	Valid %	Cumulative %
Submucosal	10	33.3	33.3	33.3
Oral	10	33.3	33.3	66.7
Intramuscular	10	33.3	33.3	100.0
Total	30	100.0	100.0	

Table 2

Mean values and variations in the variable values specified for the size of the swelling among groups during the examination period

Swelling (cm)	Groups			*p*-value between groups
	submucosal	oral	intramuscular	
1st day	13.1 ± 1.8	12.27 ± 0.8	12.88 ± 1.5	
2nd day	13.04 ± 1.8	12.26 ± 0.8	12.86 ± 1.4	0.419
7th day	12.39 ± 1.8	11.61 ± 0.7	12.2 ± 1.4	
p-value in time series		< 0.001		

Results are expressed as mean ± standard deviation.

three groups. There was no statistically significant difference in the size of swelling between the groups ($p = 0.419$) in any examination period (Table 2).

Trismus (interincisal distance)

Preoperatively, the arithmetic mean and SD of the interincisal distance was 3.9 ± 0.5 cm in the submucosal group, 4 ± 0.5 cm in the oral group, while in the intramuscular group, it was 4 ± 0.7 cm, which is not a statistically significant difference ($p = 0.787$). The distribution of patients among groups was valid.

Overall, there was a significant increase in the interincisal distance in the examination period ($p < 0.001$) among all three groups. There was no statistically significant difference in the interincisal distance between the groups ($p = 0.939$) in any examination period (Table 3).

Visual analog scale

Overall, there was a significant decrease in pain levels in the examination period ($p < 0.001$) among all three groups. There was no statistically significant difference in pain levels between the groups ($p = 0.725$) in any examination period (Table 4).

Number of analgesics

There was no statistically significant difference in the number of analgesic drugs taken by patients between the groups ($p = 0.069$) (Table 5).

Discussion

In order to prevent the occurrence of postoperative complications after surgical extraction of the impacted lower third molars, dexamethasone can be used locally, most often submucosally or systemically, intramuscularly, intravenously, or orally. Although the intravenous drugs administration provides the rapid achievement of high plasma concentrations, and thus the immediate effect, this route of the administration of dexamethasone is rarely used in outpatient settings because it is more difficult to perform, more unpleasant for the patient and has a higher rate of the possible complications after the administration. In addition, some studies show that a significant benefit cannot be achieved with the intravenous administration route compared to other routes of dexamethasone administration [9, 10].

In this study, three routes of dexamethasone administration were used to prevent postoperative complications of trismus, pain, and swelling. The analysis of the obtained results did not reveal a statistically significant difference in the effect of dexamethasone in relation to all three examined parameters of submucosal, intramuscular, or oral administration. These results can be compared with other similar studies [21–23].

The submucosal route of dexamethasone administration, locally in the buccal sulcus area, is a relatively newer route of its administration that has shown high efficacy in the prevention of the postoperative complications of surgery of the impacted lower third molars in numerous studies [24–26]. However, similar studies have not shown statistically significant benefits of this route of administration compared to oth-

Table 3

Mean values and variations of interincisal distance (trismus) among groups during the examination period

Trismus (cm)	Groups			*p*-value between groups
	submucosal	oral	intramuscular	
1st day	3.54 ± 0.5	3.32 ± 0.6	3.4 ± 0.7	
2nd day	3.57 ± 0.5	3.42 ± 0.7	3.49 ± 0.7	0.939
7th day	3.89 ± 0.5	3.98 ± 0.5	3.97 ± 0.7	
p-value in time series		< 0.001		

Results are expressed as mean ± standard deviation.

Table 4

Mean values and variations of pain levels among groups during the examination period

Visual analog scale	Groups			*p*-value between groups
	submucosal	oral	intramuscular	
1st day	3 (range, 1–7)	4 (range, 3–8)	3.5 (range, 1–7)	
2nd day	2.5 (range, 0–8)	3 (range, 1–8)	4 (range, 0–7)	0.725
7th day	0 (range, 0–1)	0 (range, 0–2)	0 (range, 0–2)	
p-value in time series		< 0.001		

Table 5

Total number of analgesics consumed during the examination period according to the route of administration

Parameter	Groups		
	submucosal	oral	intramuscular
Number of analgesics	4 (range, 1–8)	6.5 (range, 2–10)	4.5 (range, 2–6)
p-value between groups		0.069	

ers. In their study, Noboa et al. [21] state that the effect of the submucosal administration of dexamethasone does not show a statistically significant difference in the prevention of these complications compared to the oral route of administration. Although it is a systemic route of administration in relation to the local application, this can be explained by the fact that the resorption of dexamethasone in the digestive tract is very good, followed by its very high concentration in plasma.

In a comparative analysis of the effect of dexamethasone administration on the degree of postoperative complications, Majid and Mahmood [22] did not notice a statistically significant difference between submucosal and intramuscular administration. Likewise, Gopalakrishnan et al. [23] did not notice in their analysis a difference in the prevention of trismus, pain, and swelling *via* the intramuscular route of administration compared to the submucosal route of dexamethasone administration. On the other hand, Antunes et al. [27] have examined the effects of intramuscular and oral administrations of dexamethasone and did not find a statistically significant difference. Boonsiriseth et al. [28] conducted almost the same study with the same conclusion. The only difference between these two studies is that for the intramuscular application of dexamethasone, the area of the masseter muscle was used in the first one, and in the second one, the area of the deltoid muscle was used. That may indirectly indicate that intramuscular administration of dexamethasone at local and remote sites has the same effect.

Considering the results of this study and similar studies of other authors, it can be said that the use of corticosteroids, especially dexamethasone, leads to a significant drop in swelling, trismus, and pain after the surgical extraction of the impacted lower third molars. In addition, the route of administration of dexamethasone does not lead to a statistically significant difference when it comes to the prevention of mentioned complications. Choosing the route of administration depends exclusively on the affinity of the therapist as well as the characteristics of each patient as an individual. The intramuscular route of administration, although undoubtedly effective, according to the authors of this study, causes moderate discomfort in the form of pain or tingling at the site of the application when administered in the area of the deltoid muscle. In addition, this route of administration is particularly inconvenient for patients suffering from symptoms of trypanophobia. Finally, the intramuscular route of administration may be problematic in some patients for religious and cultural reasons. All these disadvantages can be avoided by oral or submucosal administration of dexamethasone. However, the oral route, due to the mode of absorption and the need to achieve the optimal concentration of the drug in the blood during and after the operation, requires administration usually one hour before the beginning of the intervention. In addition, the minimum effective dose of dexamethasone mentioned in the literature that should be used in order to prevent postoperative complications is 4 mg, depending on the manufacturer. That requires taking more tablets at the same time, which in some cases, causes confusion and doubt. According to our experience, the submucosal route of administration can be considered the most appropriate because the therapeutic dose can be achieved using only one ampoule of dexamethasone. In addition, for people suffering from needle phobia, this route of administration is more acceptable than the intramuscular; it causes no sensations because the medication is applied submucosally in the buccal sulcus region of the impacted lower third molars after the local anesthesia has already been given.

Limitations of the study

The main limitation of this study might be an insufficient sample size that could be enlarged in future studies.

Conclusion

Within the limitations of this study, it can be concluded that there is no significant difference in dexamethasone effectiveness in postoperative trismus, swelling, and pain reduction after the third molar surgery, regarding the route of administration – oral, intramuscular, or local submucosal.

REFERENCES

1. *Messer EJ, Keller JJ.* The use of intraoral dexamethasone after extraction of mandibular third molars. Oral Surg Oral Med Oral Pathol 1975; 40(5): 594–8.
2. *Beirne OR, Hollander B.* The effect of methylprednisolone on pain, trismus, and swelling after removal of third molars. Oral Surg Oral Med Oral Pathol 1986; 61(2): 134–8.
3. *McGrath C, Comfort MB, Lo EC, Luo Y.* Changes in life quality following third molar surgery – the immediate postoperative period. Br Dent J 2003; 194(5): 265–8; discussion 261.
4. *Alexander RE, Throndson RR.* A review of perioperative corticosteroid use in dentoalveolar surgery. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000; 90(4): 406–15.
5. *Markiewicz MR, Brady MF, Ding EL, Dodson TB.* Corticosteroids reduce postoperative morbidity after third molar surgery: a systematic review and meta-analysis. J Oral Maxillofac Surg 2008; 66(9): 1881–94.
6. *Kim K, Brar P, Jakubowski J, Kaltman S, Lopez E.* The use of corticosteroids and nonsteroidal antiinflammatory medication for the management of pain and inflammation after third molar surgery: a review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009; 107(5): 630–40.
7. *Shaikh S, Verma H, Yadav N, Jauhari M, Bullangowda J.* Applications of steroid in clinical practice: a review. ISRN Anesthesiology 2012; 2012: 1–11.
8. *Darwade DA, Kumar S, Mehta R, Sharma AR, Reddy GS.* In search of a better option: dexamethasone versus methylprednisolone in third molar impaction surgery. J Int Oral Health 2014; 6(6): 14–7.
9. *Majid OW, Mahmood WK.* Use of dexamethasone to minimise post-operative sequelae after third molar surgery: comparison of five different routes of administration. Oral Surg 2013; 6(4): 200–8.
10. *Chaudhary PD, Rastogi S, Gupta P, Niranjanaprasad Indra B, Thomas R, Choudhury R.* Pre-emptive effect of dexamethasone injection and consumption on post-operative swelling, pain, and trismus after third molar surgery. A prospective, double

blind and randomized study. J Oral Biol Craniofac Res 2015; 5(1): 21–7.
11. *Nehme W, Fares Y, Abou-Abbas L.* Piezo-surgery technique and intramuscular dexamethasone injection to reduce postoperative pain after impacted mandibular third molar surgery: a randomized clinical trial. BMC Oral Health 2021; 21(1): 393.
12. *Falci SGM, Lima TC, Martins CC, Santos CRRD, Pinheiro MLP.* Preemptive Effect of Dexamethasone in Third-Molar Surgery: A Meta-Analysis. Anesth Prog 2017; 64(3): 136–43.
13. *Alcântara CE, Falci SG, Oliveira-Ferreira F, Santos CR, Pinheiro ML.* Pre-emptive effect of dexamethasone and methylprednisolone on pain, swelling, and trismus after third molar surgery: a split-mouth randomized triple-blind clinical trial. Int J Oral Maxillofac Surg 2014; 43(1): 93–8.
14. *Zerener T, Aydintug YS, Sencimen M, Bayar GR, Yazici M, Altug HA,* et al. Clinical comparison of submucosal injection of dexamethasone and triamcinolone acetonide on postoperative discomfort after third molar surgery. Quintessence Int 2015; 46(4): 317–26.
15. *Ngeow WC, Lim D.* Do Corticosteroids Still Have a Role in the Management of Third Molar Surgery? Adv Ther 2016; 33(7): 1105–39.
16. *Graziani F, D'Aiuto F, Arduino PG, Tonelli M, Gabriele M.* Perioperative dexamethasone reduces post-surgical sequelae of wisdom tooth removal. A split-mouth randomized double-masked clinical trial. Int J Oral Maxillofac Surg 2006; 35(3): 241–6.
17. *Grossi GB, Maiorana C, Garramone RA, Borgonovo A, Beretta M, Farronato D*, et al. Effect of submucosal injection of dexamethasone on postoperative discomfort after third molar surgery: a prospective study. J Oral Maxillofac Surg 2007; 65(11): 2218–26.
18. *Pell GJ, Gregory GT.* Impacted mandibular third molar: Classification and modified technique for removal. Dent Dig 1933; 39(9): 330–8.
19. *Winter GB.* Impacted mandibular third molar. St. Louis: American Medical Book; 1926.
20. *Schultze-Mosgau S, Schmelzeisen R, Frölich JC, Schmele H.* Use of ibuprofen and methylprednisolone for the prevention of pain and swelling after removal of impacted third molars. J Oral Maxillofac Surg 1995; 53(1): 2–7; discussion 7–8.
21. *Noboa MM, Ramacciato JK, Teixeira RG, Viccentini CB, Groppo CG, Motta RH.* Evaluation of effects of two dexamethasone formulations in impacted third molar surgeries. Rev Dor São Paulo 2014; 15(3): 163–8.
22. *Majid OW, Mahmood WK.* Effect of submucosal and intramuscular dexamethasone on postoperative sequelae after third molar surgery: comparative study. Br J Oral Maxillofac Surg 2011; 49(8): 647–52.
23. *Gopalakrishnan V, Darekar HS, Sahoo NK.* Effectiveness of submucosal v/s intramuscular dexamethasone in mandibular third molar surgeries. Int J Med Sci Clin Inven 2015; 2(1): 648–55.
24. *Nair RB, Rahman NM, Ummar M, Hafiz KA, Issac JK, Sameer KM.* Effect of submucosal injection of dexamethasone on postoperative discomfort after third molar surgery: a prospective study. J Contemp Dent Pract 2013; 14(3): 401–4.
25. *Ehsan A, Ali Bukhari SG, Ashar, Manzoor A, Junaid M.* Effects of pre-operative submucosal dexamethasone injection on the postoperative swelling and trismus following surgical extraction of mandibular third molar. J Coll Physicians Surg Pak 2014; 24(7): 489–92.
26. *Warraich R, Faisal M, Rana M, Shaheen A, Gellrich NC, Rana M.* Evaluation of postoperative discomfort following third molar surgery using submucosal dexamethasone - a randomized observer blind prospective study. Oral Surg Oral Med Oral Pathol Oral Radiol 2013; 116(1): 16–22.
27. *Antunes AA, Avelar RL, Martins Neto EC, Frota R, Dias E.* Effect of two routes of administration of dexamethasone on pain, edema, and trismus in impacted lower third molar surgery. Oral Maxillofac Surg 2011; 15(4): 217–23.
28. *Boonsiriseth K, Klongnoi B, Sirintawat N, Saengsirinavin C, Wongsirichat N.* Comparative study of the effect of dexamethasone injection and consumption in lower third molar surgery. Int J Oral Maxillofac Surg 2012; 41(2): 244–7.

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Effect of *Staphylococcus aureus* in experimental pneumonia mouse model on promotion of mBD-3 expression through activation of the ERK1/2 pathway

Uticaj *Staphylococcus aureus*-a na porast ekspresije mBD-3 posredovan aktivacijom ERK1/2 signalnog puta na eksperimentalnom modelu mišje pneumonije

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Abstract

Background/Aim. *Staphylococcus aureus (S. aureus)* is a gram-positive pathogen that causes various human diseases. *S. aureus* causes pneumonia, which is characterized by localized tissue necrosis. The aim of the study was to explore the expression of mouse β-defensin 3 (mBD-3) induced by *S. aureus* in mouse lungs and the effect of mBD-3 expression on the mitogen-activated protein kinase (MAPK) pathway. **Methods.** An experimental model of *S. aureus* pneumonia in mice was developed, and the expression of mBD-3 and activation of the MAPK pathway were investigated using the methods of immunofluorescence and western blot. **Results.** The experimental model was created successfully. The number of white blood cells was elevated 48 and 72 hrs after the introduction of bacteria through mouse airways, and bronchiolar mucosal hyperemia was observed, along with a large number of white blood cells and mucus in the bronchioles. The mBD-3 expression levels 48 and 72 hrs after the induction of infection were greater than the levels in the control group and 24 hrs after the induction. The amount of phosphorylated extracellular signal-regulated kinase (ERK1/2) was increased 48 and 72 hrs after infection induction, compared with the levels in the control group and 24 hrs after induction. The expression of mBD-3 was lower when ERK1/2 phosphorylation was inhibited by the U0126 inhibitor. **Conclusion.** *S. aureus* in experimental pneumonia mouse model accelerates mBD-3 expression in the mouse lung mainly through an ERK1/2-dependent signaling pathway.

Key words:
defensins; disease models, animal; pneumonia, staphylococcal; signal transduction; staphylococcus aureus.

Apstrakt

Uvod/Cilj. *Staphylococcus aureus (S. aureus)* je gram-pozitivni patogen koji izaziva različite bolesti kod ljudi. *S. aureus* izaziva upalu pluća, koju karakteriše lokalizovana nekroza tkiva. Cilj rada bio je da se ispita ekspresija mišjeg β-defensina 3 (mBD-3) izazvana *S. aureus*-om u plućima miša i efekat ekspresije mBD-3 na signalni put mitogenom-aktivisanih protein kinaza (MAPK). **Metode.** Razvijen je eksperimentalni model pneumonije izazvane *S. aureus*-om kod miševa, a ekspresija mBD-3 i aktivacija MAPK signalnog puta ispitivana je metodama imunofluorescencije i *western blot*-a. **Rezultati.** Eksperimentalni model je uspešno kreiran. Broj belih krvnih zrnaca bio je povećan nakon 48 i 72 sata od unosa bakterija kroz disajne puteve miševa, a uočena je hiperemija bronhiolarne sluzokože uz prisustvo velikog broja belih krvnih zrnaca i sluzi u bronhiolama. Nivoi ekspresije mBD-3 nakon 48 i 72 sata od indukcije infekcije bili su viši od nivoa u kontrolnoj grupi i nakon 24 sata od indukcije. Količina fosforilisane kinaze regulisane ekstracelularnim signalom (*extracellular signal-regulated kinase*, ERK1/2) bila je povećana nakon 48 i 72 sata posle indukcije infekcije u poređenju sa nivoom u kontrolnoj grupi i 24 sata posle indukcije. Ekspresija mBD-3 bila je niža kada je fosforilacija ERK1/2 bila inhibirana primenom inhibitora U0126. **Zaključak.** *S. aureus* na eksperimentalnom modelu mišje pneumonije ubrzava ekspresiju mBD-3 u plućima miša uglavnom posredstvom ERK1/2-zavisnog signalnog puta.

Ključne reči:
defensini; bolest, modeli na životinjama; pneumonija, stafilokokna; signali, transdukcija; staphylococcus aureus.

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Introduction

Staphylococcus aureus (*S. aureus*) is an important gram-positive human pathogen responsible for various diseases and represents a leading cause of pneumonia (*S. aureus* pneumonia); its importance as a respiratory pathogen is on the rise [1, 2]. *S. aureus* pneumonia, characterized by localized necrosis and inflammation, is one of the most prevalent *S. aureus*-mediated diseases and occurs in nearly 13.3% of all invasive staphylococcal infections [3]. However, because of the ability of *S. aureus* to develop resistance to a large range of antibiotics, a decrease in the efficiency of current treatments has been reported. Therefore, the development of novel antibiotics or therapeutic strategies against staphylococcal infections is an obvious requirement [4, 5].

Defensins are endogenous cationic peptides and effector molecules for the immune system because of their broad-spectrum antimicrobial activity [6, 7]; they are classified into α and β subfamilies. β-defensins exhibit a broad spectrum of activities against bacteria, viruses, and fungi, particularly at the epithelial interface of mucosal surfaces. β-defensin 3 (BD-3) plays an important role in the inhibition of bacterial infections [8, 9]. In humans, BD-3 has been found in airway surface fluids from patients with psoriasis, suggesting the protein may play a role in fighting local infection [10, 11]. Human BD-3 can also enhance resistance against influenza virus and bacterial infections [12]. Mouse BD-3 (mBD-3) has the same functions as human BD-3 [13].

Mitogen-activated protein kinases (MAPKs) constitute a paradigm of intracellular signaling [14]. They are expressed ubiquitously and control a wide variety of critical cellular functions such as proliferation, differentiation, migration, and apoptosis [15]. In this study, mBD-3 expression in mouse epithelial cells was induced by *S. aureus* in mouse lungs, and the effect of mBD-3 expression on the MAPK pathway was explored.

Methods

Experimental model of S. aureus pneumonia

All procedures for the animal experiment were approved by the Animal Experimental Ethics Committee of Inner Mongolia Medical University (No. YKD2020059), and all protocols complied with relevant guidelines and the Animal Research Guideline. Specific pathogen-free mice were used for *S. aureus* infection experiments at seven weeks of age. The mice were divided into two groups (the control group and the *S. aureus* pneumonia group). The *S. aureus* pneumonia group mice were inoculated with 100 μL of *S. aureus (*ATCC-25923, 10^9 cells/mL) via the nasal route [16–19]. The blood and lung tissue samples were collected 24, 48, and 72 hrs after induction of pneumonia, and the white blood cell counts were analyzed with an Veterinary hematology analyzer (MEK-6450K, NIHON KOHDEN). Mice were humanely sacrificed by euthanasia (chloral hydrate) after treatment.

Hematoxylin and eosin staining

The lung tissue samples were fixed with 10% neutral buffered formalin, embedded in paraffin, and cut into 4 μm thin pieces. The sections were deparaffinized at 65 °C for 4 hrs with gradient ethanol and then stained with hematoxylin or Masson's trichrome staining.

Cell culture

Pulmonary epithelial cells were obtained from the lungs of suckling mice [20–22]. The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin-streptomycin in a 37 °C incubator with an atmosphere of 5% CO_2 and 95% air. *S. aureus* (10^3 cells/well) was added to the pulmonary epithelial cells (10^6 cells/well), and then the cells were incubated for 4, 8, and 12 hrs, and the inhibitor U0126 (1.5 μM) was added to evaluate the function of phosphorylated ERK1/2.

Immunofluorescence

The lung tissue was frozen in liquid nitrogen and placed in a constant-temperature freezer. A small amount of optimal cutting temperature compound was added for cryosectioning. The sections were dried using a cold air blower and blocked with 5% bovine serum albumin for one hour. Afterward, the frozen sections were incubated with specific antibodies against mBD-3, washed, and labeled with Alexa Fluor 488 goat anti-rabbit IgG (Invitrogen, A11008) for one hour. Finally, the samples were imaged and analyzed with fluorescence microscopy (Leica).

The pulmonary epithelial cells grown on poly-L-lysine (Biochrom AG Seromed) coated glass coverslips were fixed with methanol/acetone. The fixed cells were incubated with mBD-3 antibody for one hour at room temperature (RT), followed by goat anti-rabbit IgG conjugated to Alexa Fluor 488 for one hour at RT. Finally, the samples were imaged and analyzed with fluorescence microscopy (Leica).

Western blot

The lung tissue or pulmonary epithelial cells were washed twice with cold PBS and lysed with radioimmunoprecipitation lysis buffer (containing a protease inhibitor cocktail). The whole cell lysates were incubated at 4 °C for 30 min, followed by centrifugation (12,000 g for 15 min, at 4 °C). Protein samples were separated with SDS-PAGE electrophoresis, transferred to a polyvinylidene difluoride (PVDF) membrane, and probed with specific antibodies against anti-ERK1/2 (Millipore Cat. No. 16-284), anti-phospho-ERK1/2 (Millipore Cat. No. 05-797), anti-c-Jun N-terminal kinase (JNK) (Millipore Cat. No. 04-210), anti-phospho-JNK (Millipore Cat. No. 46-613MAG), anti-p38 kinase (Millipore Cat. No. ABS29), and anti-phospho-p38 (Millipore Cat. No. MABS64).

Statistical analysis

Numerical data were analyzed as the mean ± standard deviation (SD) and compared using the unpaired Student's *t*-test. Differences in values were considered significant at $p < 0.05$.

Results

Effect of S. aureus on white blood cell count and lung tissue

The white blood cell count in the *S. aureus*-infected group was higher than in the control group ($p < 0.05$). Additionally, the white blood cell count was greater after 48 and 72 hrs than after 24 hrs of pneumonia induction ($p < 0.05$). However, there was no significant difference between the numbers 48 and 72 hrs after induction of the infection (Figure 1A).

In the lung tissue stained with hematoxylin and eosin, the presence of more white blood cells corresponded with more bronchiolar mucosal hyperemia 48 and 72 hrs after induction of the infection, and mucus was observed in the bronchioles, unlike the results for the control group and 24 hrs after induction of the infection (Figure 1B).

Expression of mBD-3 and the MAPK pathway in lung tissue

To determine the expression of mBD-3 in the lung tissue, proteins were extracted from the lungs of *S. aureus* pneumonia mice. The results showed that the mBD-3 level of expression was higher 48 and 72 hrs after pneumonia induction than the corresponding expression in the control group and 24 hrs after pneumonia induction (green Figure 2).

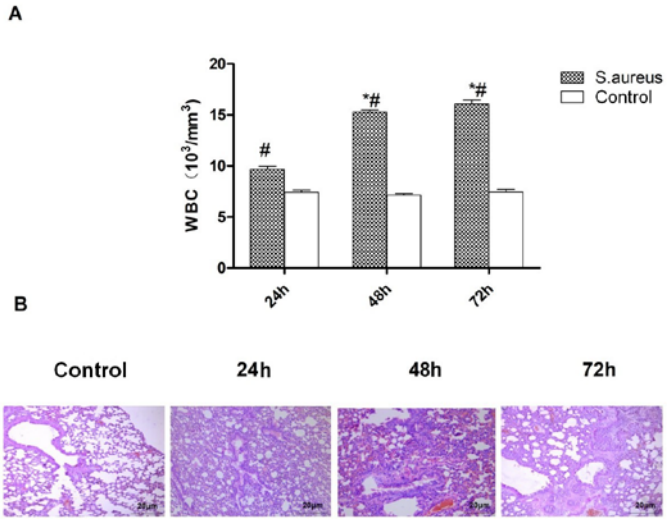

Fig. 1 – A) The white blood cells (WBC) count; B) Hematoxylin and eosin staining of lung tissue from *Staphylococcus aureus* pneumonia mice. * significantly different from the 24-hour data point ($p < 0.05$); # significantly different from the control group at the same time point ($p < 0.05$).

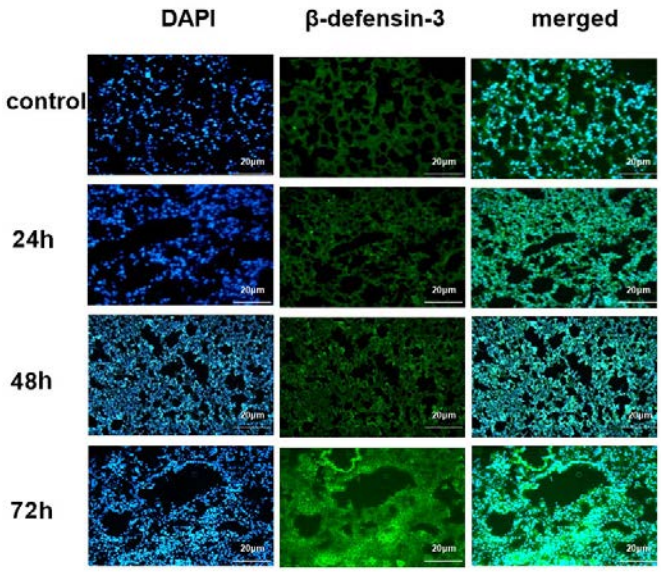

Fig. 2 − Mouse β-defensin 3 expression in lung tissue of *Staphylococcus aureus* pneumonia mice determined by immunofluorescence. DAPI- 4′,6-diamidino-2-phenylindole staining.

To assess MAPK signaling activity, the levels of total and phosphorylated ERK1/2, JNK, and p38 were measured by western blot (Figures 3A and B). The abundance of phosphorylated ERK1/2 was increased 48 and 72 hrs after pneumonia induction compared with the levels in the control group and 24 hrs after the induction (Figure 3B, $p < 0.05$). However, there were no changes in other proteins. Therefore, ERK1/2 may be the key protein that regulates mBD-3 expression.

Expression of mBD-3 and the MAPK pathway in pulmonary epithelial cells

Pulmonary epithelial cells incubated with *S. aureus* were evaluated for the expression of mBD-3 and the changes in the MAPK pathway. The results showed that the expression of mBD-3 in the pulmonary epithelial cells increased as the length of exposure to *S. aureus* increased (green Figure 4A). To assess the MAPK signaling activity in pulmonary

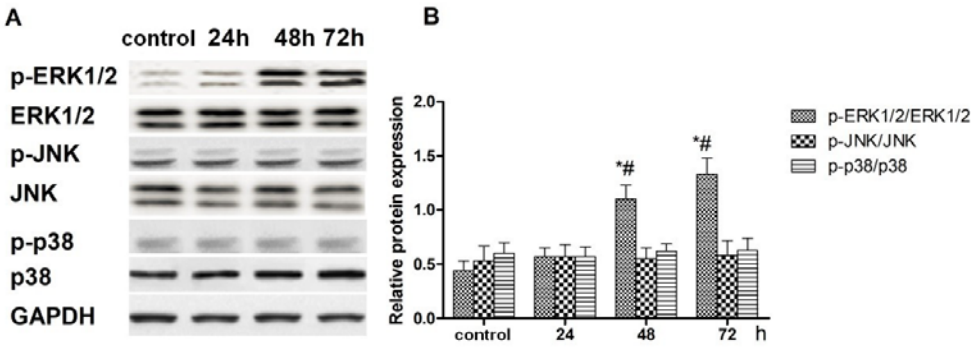

Fig. 3 − Effect of *Staphylococcus aureus* on mitogen-activated protein kinase pathway in lung tissue: A) Representative western blots showing levels of ERK1/2, JNK, p38, p-ERK1/2, p-JNK, p-p38, and GAPDH; B) Histograms summarizing the results shown in A); GAPDH – glyceraldehyde-3-phosphate dehydrogenase; ERK1/2 – extracellular signal-regulated kinase 1/2; JNK – c-Jun N-terminal kinase; p38 – p38 kinase; p-ERK1/2 – phosphorylated ERK 1/2; p-JNK – phosphorylated JNK; p-p38 – phosphorylated p38. Results are expressed as mean ± standard deviation (n = 5); * significantly different from the control group ($p < 0.05$); # significantly different from the 24-hour data point ($p < 0.05$).

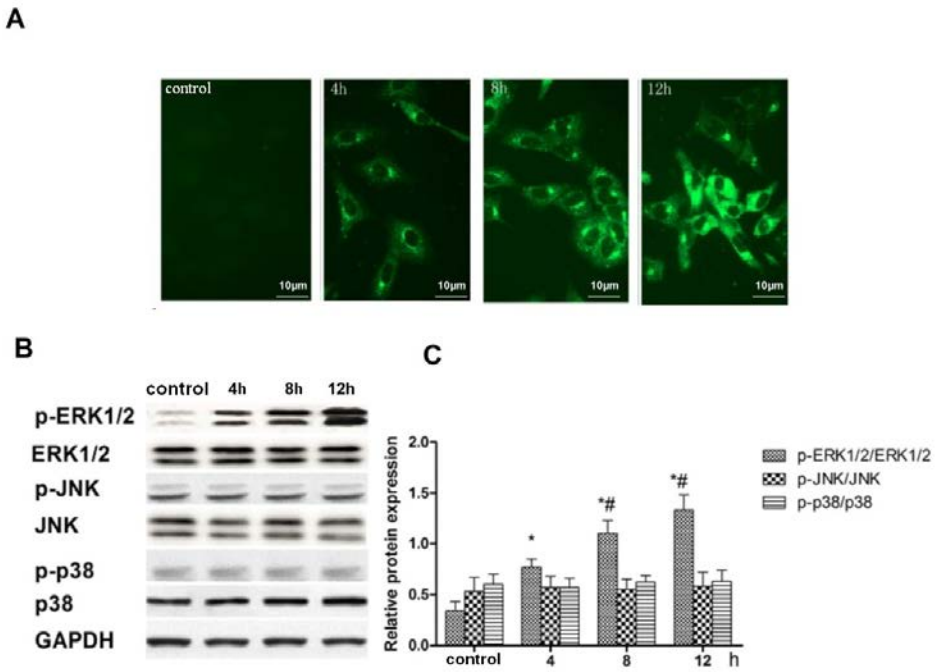

Fig. 4 − Effect of *Staphylococcus aureus* on mouse β-defensin 3 expression and mitogen-activated protein kinase pathway in pulmonary epithelial cells: A) mBD-3 expression determined by immunofluorescence; B) Representative western blots showing levels of ERK1/2, p38, p-ERK1/2, p-JNK, p-p38, and GAPDH; C) Histograms summarizing the results shown in B). For abbreviations see under Figure 3.
Results are expressed as mean ± standard deviation (n = 5); * significantly different from the control group ($p < 0.05$); # significantly different from the 4-hour data point ($p < 0.05$).

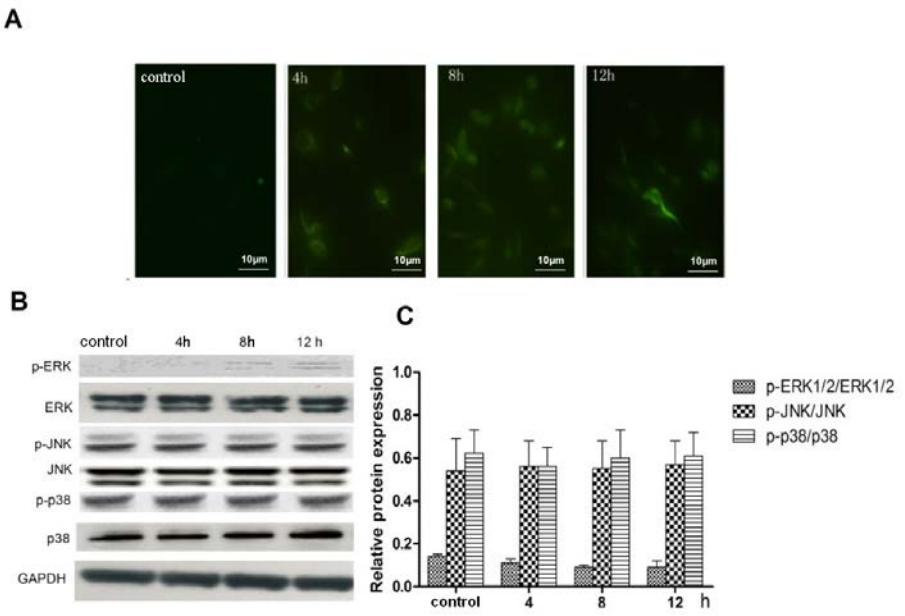

Fig. 5 − Effect of *Staphylococcus aureus* on mouse β-defensin 3 expression and mitogen-activated protein kinase pathway after pERK1/2 inhibition in pulmonary epithelial cells: A) mBD-3 expression determined by immunofluorescence; B) Representative western blots showing levels of ERK1/2, JNK, p38, p-ERK1/2, p-JNK, p-p38, and GAPDH; C) Histograms summarizing the results shown in B). For abbreviations see under Figure 3. Results are expressed as mean ± standard deviation (n = 5).

epithelial cells, the levels of total and phosphorylated ERK1/2, JNK, and p38 kinase were measured by western blot analysis 4, 8, and 12 hrs after the beginning of incubation. The phosphorylated ERK1/2 was highest at 8- and 12-hour points (Figures 4B and C, $p < 0.05$), suggesting that ERK1/2 plays a key role in mBD-3 expression. To test this theory, U0126 (a specific inhibitor of ERK1/2 phosphorylation) was used to silence ERK1/2 expression. The result showed a decrease in the expression of mBD-3 (Figure 5A) when ERK1/2 phosphorylation was inhibited, while the expression of other proteins remained unchanged (Figures 5B and C).

Discussion

S. aureus is a globally distributed pathogen that can induce serious diseases in many species and cause infections in lung tissue, soft tissue, the bloodstream, etc. [13, 23]. Since mBD-3 is a natural antimicrobial peptide, it can inhibit the growth of bacteria and viruses. An experimental model of *S. aureus* pneumonia in mice was successfully developed [24, 25], and in this study the effect of mBD-3 expression on the MAPK pathway was explored.

The white blood cell count was higher 48 and 72 hrs after induction of infection compared to the control group and the 24-hour point. Bronchiolar mucosal hyperemia was observed, along with the presence of a large number of white blood cells and mucus in the bronchioles. *S. aureus* infection was found in the lungs.

In the lungs of mice with *S. aureus* pneumonia, the mBD-3 expression was higher, and the level of phosphorylated ERK1/2 was increased 48 and 72 hrs after the onset of infection in comparison to the 24 hrs after the onset of infection. That indicated that the levels of mBD-3 expression and phosphorylated ERK1/2 increased over time. We also evaluated the activity of MAPK signaling pathways. MAPKs are a superfamily of serine/threonine kinases that includes ERK1/2, JNK, and p38 [26–28]. These kinases are involved primarily in the activation of nuclear transcription factors that control cell proliferation, differentiation, and apoptosis. Our results suggest that *S. aureus* accelerates mBD-3 expression via the ERK1/2 signaling pathway, not through the activation of JNK or p38. The level of phosphorylated ERK1/2 in pulmonary epithelial cells exposed to *S. aureus* was highest 8 and 12 hrs after the beginning of incubation with the bacteria. Furthermore, the blocking of ERK1/2 by chemical inhibition suppressed mBD-3 expression. When *S. aureus* invaded the lung epithelial cells, the mBD-3 secreted from the cells enhanced the resistance against infection, and the phosphorylated ERK1/2 promoted mBD-3 secretion.

Conclusion

Overall, our results provide evidence that the *S. aureus* signaling pathway accelerates mBD-3 expression mainly through an ERK1/2-dependent pathway in mouse lungs. Thus, our findings suggest that mBD-3 expression is regulated by the ERK1/2 pathway.

Funding

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R E F E R E N C E S

1. *Fode P, Stegger M, Andersen PS*. Human β-defensin 3 (DEFB103) and its influence on Staphylococcus aureus nasal carriage. Int J Infect Dis 2011; 15(6): e388–94.
2. *Kisich KO, Howell MD, Boguniewicz M, Heizer HR, Watson NU, Leung DY*. The constitutive capacity of human keratinocytes to kill Staphylococcus aureus is dependent on beta-defensin 3. J Invest Dermatol 2007; 127(10): 2368–80.
3. *Wu Q, Gui P, Yao S, Zhu H, Li J, Li Y*. Expression of β-defensin-3 in lungs of immunocompetent rats with methicillin-resistant Staphylococcus aureus ventilator-associated pneumonia. J Surg Res 2011; 169(2): 277–83.
4. *Pascoal MA, Marson FAL, Paschoal IA, Levy CE*. Influence of pancreatic status, CFTR mutations, Staphylococcus aureus and/or Pseudomonas aeruginosa infection/colonization on lung function in cystic fibrosis during a 2-year follow-up period. Wien Klin Wochenschr 2020; 132(19–20): 572–80.
5. *Wu Y, Nie Y, Huang J, Qiu Y, Wan B, Liu G*, et al. Protostemonine alleviates heat-killed methicillin-resistant Staphylococcus aureus-induced acute lung injury through MAPK and NF-κB signaling pathways. Int Immunopharmacol 2019; 77: 105964.
6. *Mücke PA, Ostrzinski A, Hammerschmidt S, Maaß S, Becher D*. Proteomic Adaptation of Streptococcus pneumoniae to the Antimicrobial Peptide Human Beta Defensin 3 (hBD3) in Comparison to Other Cell Surface Stresses. Microorganisms 2020; 8(11): 1697.
7. *Sechet E, Telford E, Bonamy C, Sansonetti PJ, Sperandio B*. Natural molecules induce and synergize to boost expression of the human antimicrobial peptide β-defensin-3. Proc Natl Acad Sci U S A 2018; 115(42): E9869–78.
8. *Chen L, Lv Z, Gao Z, Ge G, Wang X, Zhou J,* et al. Human β-defensin-3 reduces excessive autophagy in intestinal epithelial cells and in experimental necrotizing enterocolitis. Sci Rep 2019; 9(1): 19890.
9. *Priyadharshini VS, Ramírez-Jiménez F, Molina-Macip M, Renteria-Rosales C, Santiago-Cruz J, Zarate-Segura P,* et al. Human Neutrophil Defensin-1, -3, and -4 Are Elevated in Nasal Aspirates from Children with Naturally Occurring Adenovirus Infection. Can Respir J 2018; 2018: 1038593.
10. *Dhingra H, Kaur K, Singh B*. Engineering and characterization of human β-defensin-3 and its analogues and microcin J25 peptides against Mannheimia haemolytica and bovine neutrophils. Vet Res 2021; 52(1): 83.
11. *He W, Wei D, Zhang J, Huang X, He D, Liu B,* et al. Novel bone repairing scaffold consisting of bone morphogenetic Protein-2 and human Beta Defensin-3. J Biol Eng 2021; 15(1): 5.
12. *Cooper CA, Tizard ML, Stanborough T, Moore SC, Chandry PS, Jenkins KA*, et al. Overexpressing ovotransferrin and avian β-defensin-3 improves antimicrobial capacity of chickens and poultry products. Transgenic Res 2019; 28(1): 51–76.
13. *Zhang Y, Zhao Y, Liu H, Yu W, Yang F, Li W*, et al. Mouse β-Defensin 3, A Defensin Inhibitor of Both Its Endogenous and Exogenous Potassium Channels. Molecules 2018; 23(6): 1489.
14. *Wang Z, Zhu M, Wang M, Gao Y, Zhang C, Liu S*, et al. Integrated Multiomic Analysis Reveals the High-Fat Diet Induced Activation of the MAPK Signaling and Inflammation Associated Metabolic Cascades via Histone Modification in Adipose Tissues. Front Genet 2021; 12: 650863.
15. *Okkenhaug K*. How to resist PI3Kδ inhibition: activate MAPK! Blood 2021; 138(1): 3–4.
16. *Wang L, Li Q, Li J, Jing S, Jin Y, Yang L,* et al. Eriodictyol as a Potential Candidate Inhibitor of Sortase A Protects Mice From Methicillin-Resistant Staphylococcus aureus-Induced Pneumonia. Front Microbiol 2021; 12: 635710.
17. *Meijer MT, de Vos AF, Peters Sengers H, Scicluna BP, Roelofs JJ, Abou Fayçal C,* et al. Tenascin C Has a Modest Protective Effect on Acute Lung Pathology during Methicillin-Resistant Staphylococcus aureus-Induced Pneumonia in Mice. Microbiol Spectr 2021; 9(1): e0020721.
18. *Liu ZZ, Yang YJ, Zhou CK, Yan SQ, Ma K, Gao Y,* et al. STING Contributes to Host Defense Against Staphylococcus aureus Pneumonia Through Suppressing Necroptosis. Front Immunol 2021; 12: 636861.
19. *Murphy AG, O'Keeffe KM, Lalor SJ, Maher BM, Mills KH, McLoughlin RM*. Staphylococcus aureus infection of mice expands a population of memory γδ T cells that are protective against subsequent infection. J Immunol 2014; 192(8): 3697–708.
20. *Nayak PS, Wang Y, Najrana T, Priolo LM, Rios M, Shaw SK*, et al. Mechanotransduction via TRPV4 regulates inflammation and differentiation in fetal mouse distal lung epithelial cells. Respir Res 2015; 16(1): 60.
21. *Sinha M, Lowell CA*. Immune Defense Protein Expression in Highly Purified Mouse Lung Epithelial Cells. Am J Respir Cell Mol Biol 2016; 54(6): 802–13.
22. *Lam HC, Choi AM, Ryter SW*. Isolation of mouse respiratory epithelial cells and exposure to experimental cigarette smoke at air liquid interface. J Vis Exp 2011; (48): 2513.
23. *Wu S, Huang J*. Resveratrol alleviates Staphylococcus aureus pneumonia by inhibition of the NLRP3 inflammasome. Exp Ther Med 2017; 14(6): 6099–104.
24. *Jiang Y, Wang Y, Wang B, Yang D, Yu K, Yang X,* et al. Antifungal activity of recombinant mouse beta-defensin 3. Lett Appl Microbiol 2010; 50(5): 468–73.
25. *Lee SE, Lee JS, Kim MR, Kim MY, Kim SC*. Topical retinoids induce β-defensin 3 expression in mouse skin. Int J Dermatol 2010; 49(9): 1082–4.
26. *Li J, Liu X, Wang Q, Huangfu J, Schuman MC, Lou Y*. A Group D MAPK Protects Plants from Autotoxicity by Suppressing Herbivore-Induced Defense Signaling. Plant Physiol 2019; 179(4): 1386–401.
27. *Griukova A, Deryabin P, Sirotkina M, Shatrova A, Nikolsky N, Borodkina A*. P38 MAPK inhibition prevents polybrene-induced senescence of human mesenchymal stem cells during viral transduction. PLoS One 2018; 13(12): e0209606.
28. *An Z, Huang X, Zheng C, Ding W*. Acinetobacter baumannii outer membrane protein A induces HeLa cell autophagy via MAPK/JNK signaling pathway. Int J Med Microbiol 2019; 309(2): 97–107.

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Assessment of efficacy of platelet-rich plasma application in regeneration of the facial nerve in rabbits

Procena efikasnosti primene plazme obogaćene trombocitima u procesu regeneracije facijalnog nerva kunića

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Abstract

Background/Aim. The injuries of the facial nerve lead to paralysis of the mimic musculature, which is conditioned by functional disorders accompanied by deformity of varying degrees depending on the intensity and location of the injury. Surgical treatment is a method of choice to treat an injured nerve. Injuries in the parotid lodge area are repaired by direct neurosuture in combination with platelet-rich plasma (PRP). **Methods.** The experimental study was carried out on 48 chinchilla male rabbits (*Oryctolagus cuniculus*), of about the same weight (2,500–3,000 gr), aged between 3 and 4 months in two surgical stages, in two different periods – six and ten weeks after the first surgical procedure. The animals were divided into four groups: Group I (suture); Group II [suture and fibrin glue (FG)]; Group III (suture and PRP); Group IV (sutures, FG, and PRP). Each group had two subgroups based on the duration of the experiment (six and ten weeks). A part of the dissected nerve in the length of 5 mm was subjected to histologic verification, where the number of axons and Schwann cells was determined and expressed numerically based on the histological sample of the tissue of the observed nerve. The extent of the presence of connective tissue and the degree of neovascularisation is shown by the description of histological samples by grades (connective tissue 1-4, neovascularisation 1-3). **Results.** Our results showed that all parameters of regeneration of damaged nerve showed a significantly higher regeneration efficiency after six and ten weeks of intervention in groups treated with PRP therapy with or without using FG. **Conclusion**. The use of PRP and the stimulating effect of activated growth factors results in the regeneration of the facial nerve in the sense of replication of the Schwann cells and the number of axons, with a high degree of neovascularization and minimal proliferation of connective tissue, which histologically corresponds to a healthy nerve.

Key words:
animals, laboratory; facial nerve injuries; histological techniques; nerve regeneration; platelet rich plasma; rabbit.

Apstrakt

Uvod/Cilj. Povrede facijalnog nerva dovode do paralize mimičke muskulature lica, što je uslovljeno funkcionalnim poremećajima praćenim deformitetom različitog stepena, u zavisnosti od intenziteta i lokacije povrede. Hirurško lečenje je metoda izbora za lečenje povređenog nerva. Povrede u predelu parotidne lože se saniraju primenom direktnog neurošava u kombinaciji sa plazmom bogatom trombocitima (PBT). **Metode**. Eksperimentalna studija sprovedena je na 48 činčila zečeva (*Orictolagus cuniculus*) muškog pola, približno iste težine (2 500–3 000 gr), starosti između 3 i 4 meseca, u dva hirurška zahvata, u dva različita perioda: šest i deset nedelja nakon prvog hirurškog zahvata. Životinje su podeljene u četiri grupe: Grupa I (šav); Grupa II [šav i fibrinski lepak (FL)]; Grupa III (šav i PBT); Grupa IV (šav, FL i PBT). Svaka grupa imala je po dve podgrupe na osnovu trajanja eksperimenta (šest i deset nedelja). Deo diseciranog nerva u dužini od 5 mm podvrgnut je histološkoj analizi, kojom je određen i numerički izražen broj aksona i Švanovih ćelija. Stepen prisustva vezivnog tkiva i stepen neovaskularizacije prikazani su gradacijom histoloških uzoraka (vezivno tkivo 1-4, neovaskularizacija 1-3). **Rezultati.** Svi parametri regeneracije oštećenog nerva pokazali su da je

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značajno veća efikasnost regeneracije posle šest i deset nedelja intervencije postignuta u grupama lečenim PBT terapijom, sa ili bez upotrebe FL. **Zaključak**. Upotreba PBT i stimulatorno delovanje aktiviranih faktora rasta dovodi do regeneracije facijalnog nerva, u smislu replikacije Švanovih ćelija i broja aksona, sa visokim stepenom neovaskularizacije i minimalnom proliferacijom vezivnog tkiva, što histološki odgovara zdravom nervu.

Ključne reči:
životinje, laboratorijske; n. facialis, povrede; histološke tehnike; nervi, regeneracija; plazma bogata trombocitima; zečevi.

Introduction

The facial nerve is exclusively a motor nerve with intrapetrous sensory and neurovegetative functions.

In the human body, there are 10 to 14,000 fibers (7,000 of which are motor). Injuries of the facial nerve can be intracranial, intratemporal, and extracranial. Sunderland [1] described five degrees of damage to the peripheral nerve fiber. This classification system explains the course of physiological events associated with all types of lesions that involve the facial nerve.

The result of injuries is the paralysis of the mimic muscles, defined as the complete absence of all voluntary movements in parts of the nerve field area, that causes severe esthetic and functional problems to the patient. As a special type of nerve injury, there is an iatrogenic injury most commonly caused by surgical interventions. Lacerations and contusions arise as a result of stinging wounds and blunt injuries, leading to nerve contusions with preserved continuity much less often than lacerations. Compression and stretching occur in the growth of the pathological substrate around the nerve. Burning injuries are caused by the effects of missiles and are most often localized in the temporal bone pyramid [2].

Iatrogenic damage to the facial nerve is most often caused by direct surgery. Intraoperatively, the nerve can be injured directly or indirectly [2].

Iatrogenic injuries occur as a result of numerous neurosurgical, maxillofacial (parotidectomy) [3], and otorhinolaryngeal (ORL) (mastoidectomy) [4] operations. Injuries to the facial nerve can occur when bone fractures and jaw fractures occur, when bone fragments damage or completely interrupt nerves [5]. Injuries of soft tissues in the parotid region can lead to nerve injury of varying degrees [6]. Depending on the pathological changes, the time of injuries, and the severity of damage to the facial nerve, there are several surgical procedures to be performed: decompression, neurosuture in various segments of the nerve, neuroanastomosis with other nerves (hypoglossus, glossopharyngeus) [7], as well as neuroplastic, plastic, and reconstructive surgical procedures.

Choosing the method of surgical treatment depends on the following: "the height" of the nerve lesion, the time elapsed from the moment of injury, the integrity of the distal and proximal part of the nerve, the size of the defect, the age of the patient, presence of degenerative and vascular diseases, as well as the patient's desire [2].

A direct neurosuture of the nerve can be done if the defect is less than 2 cm or 2.5 cm large. For larger facial nerve defects of 2.5 cm, in which no primary reconstruction with a nerve graft of up to 72 hrs has occurred, the nerve endpoints should be marked by the end of treatment and reconstructed after three to six weeks.

Gavron and Clemis [8] state that up to a year after the injury is a good time for hyoglossus-facial anastomosis with the "cross-face" method. Conley and Baker [9] present this problem of time imbalance from the injury and believe this method can be successfully applied no later than two years after the injury. A spontaneous or reflex function of the nerve can rarely be recovered.

Recovery of the facial nerve function is slow, while postoperative sequelae are inevitable [2].

Surgical interventions that have been used so far (epineural and fascicular neurosuture) [10] in order to recover the motor activity of the facial nerve have not fully yielded results, and further research is needed to discover new techniques for the regeneration of the injured facial nerve [11].

The possibility of using PRP growth factors in the process of nerve regeneration, which have so far been used in the regeneration of soft tissues and bones, is being considered [12].

Platelet-rich plasma (PRP) is autogenic concentrated human platelets in a small plasma volume. Platelets are natural deposits of many growth factors released from alpha granules in the form of fundamental proteins: three isomers of platelet-derived growth factor (PDGF) – PDGFαα, PDGF ββ, and PDGF αβ, two transforming growth factors (TGF) β (TGF β1 and TGF β2), vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF). Growth factors are essential for the onset of tissue repair and regeneration and have a hemostatic and anti-inflammatory effect [13, 14].

The aim of the study was to present the feasibility of using PRP in the regeneration of injured facial nerves at the experimental level, which could later be used in clinical practice.

Methods

This experiment was approved by the Ethics Committee of the Faculty of Medicine in Belgrade under number 5603/2 in March 2013. This study was conducted on 48 chinchilla male rabbits (*Oryctolagus cuniculus*) of about the same weight (2,500–3,000 gr) aged between three and four months, from the farm of the Institute of Medical Research, Military Medical Academy, Belgrade, Serbia. The pathohistological verification of definitive preparations af-

ter the completion of the experiment was done at the Institute of Pathological Anatomy of the Faculty of Veterinary Medicine, Belgrade, Serbia.

Surgical procedure

This experimental study was carried out in two surgical stages, in two different periods, six and ten weeks after the first surgical procedure, in order to monitor the optimal period of nerve regeneration. Four groups (I–IV) of six rabbits were formed, with each group divided into two subgroups depending on the time interval: six weeks (subgroup A) and ten weeks (subgroup B). The results of all four groups were compared while each animal was controlling itself.

Surgical procedures were carried out in aseptic conditions. Rabbits were anesthetized with a combination of ketamine (Ketamidor® 10% injection; 35 mg/kg i.m.), Acepromazine (Promace® injection; 0.1 mg/kg i.m.), and atropine (Atropine Sopharma®, Genotropin; 0.04 mg/kg i.m.). After that, the left half of the face and skin was disinfected with Kodan® (kodan tincture), thus creating the conditions for the first phase of the experiment.

The incision was made with a preauricular-submandibular cut and by blunt preparation through the tissue of the parotid gland identifying the facial nerve trunk directly in front of the stylomastoid foramen. The nerve resection was made before bifurcation, after which the first phase of the surgical experiment began. That implies that one of the following methods of nerve reconstruction was performed: suture – perineural microsuture (nylon 9–0) (Group I); suture and fibrin glue (FG) (Group II); suture and PRP (Group III); suture, FG, and PRP (Group IV). After completing this surgical procedure, the skin was sutured with nylon 5–0.

In the second phase, after six and ten weeks, the same surgical procedure was performed on the opposite side, and that represented the control group. On both sides, the same surgical procedures (nerve identification and nerve resection of 5 mm) were made. On the left side of the resection, there was 2 to 3 mm on both sides from the suture site. On the right side, part of the nerve was resected in the area in front of the trunk at the same length. Further procedure implied special packing of the resected parts of the nerve and carrying them to the Institute where histological analysis and preparation of PRP was done.

PRP was prepared from 5 mL of blood taken from the ear vein from each rabbit, with 0.4 mL citrate, by double centrifugation in laboratory conditions by a special technological process [14].

From 5 mL of taken blood, 0.3 mL of PRP was obtained, which was supplemented with antifibrinolytic (transemic acid 1–5 mg per 0.5 mL cryoprecipitate) and calcium chloride ($CaCl_2$) (0.05 mL 10% $CaCl_2$ per 1 mL PRP) as an activator, applied to the place where the nerve section was made. FG in the form of "Beriplast®" was used in groups II and IV. The number of platelet counts from the peripheral blood of one rabbit was 683,680 ± 186,229 × 10^3/μL, while in PRP, this number was 2,677,583 ± 1,201,418 × 10^3/μL.

The indicated components were applied directly, successively through two syringes, to the site where the nerve section was made, followed by the instantaneous conversion of the liquid into the gel. In the second phase of the experiment, six and ten weeks after the first phase, a certain portion of the nerve was dissected, which was further treated under standard conditions.

Histological analysis

After the section, parts of the nerve in the length of 5 mm were immediately fixed 24 hrs in a 4% buffered formalin solution. Then they were washed with water and dehydrated in alcohol of increasing concentration (70% to absolute), then lyophilized in xylol and molded into paraffin. Paraffin blocks were cut with a microtome on a sample thickness of 3 to 5 μm. The cross-sections were visible by hematoxylin-eosin staining. Coloring takes place according to the procedures specified in the manufacturer's instructions in several stages. Positive immunoreactivity for the S100 was recorded as nuclear staining. Our products were photographed in the form of a digital microphotograph from a digital microscope and then analyzed by the software (Imagel) program.

In terms of an objective assessment of the effectiveness of regeneration of the damaged nerve depending on the applied method, the following histological characteristics were analyzed: the number of newly created axons, degree of the presence of connective tissue, degree of neovascularization, and the number of Schwann cells.

The number of axons and Schwann cells was determined and expressed numerically based on the histological sample of the tissue of the observed nerve.

The extent of the presence of connective tissue and the degree of neovascularization was shown by the description of histological samples by grades. Connective tissue: 1 – in trace; 2 – in groups; 3 – around individual axons; 4 – around a number of axons. The degree of neovascularization: 1 – no blood vessels; 2 – individual blood vessels; 3 – blood vessels.

Statistical analysis

In the analysis of the results of the pathohistological tests, we used non-parametric tests adapted to small samples and data available in the form of ranks based on a small number of assumptions most commonly performed in practical research.

The nonparametric Kruskal-Wallis test was used to estimate the significance of group differences.

For simultaneous comparison of sample pairs within groups, the Wilcoxon test was used to determine with certainty which samples or methods of reconstructing the damaged facial nerve had a statistically significant difference in the effects.

Results

In all parameters of regeneration of the damaged nerve, our results show that after six and ten weeks of intervention, significantly higher regeneration performance was achieved in groups treated with PRP with or without using FG (Tables 1 and 2).

Comparing the results of the experimental and control groups, we obtained the following results for the indicated test parameters. On the radar diagram of effects in the median space of the results of nerve regeneration, depending on the applied method and the results measured in the control group, we note that the values closest to those in the control group record the methods of applying PRP with or without using FG, which speaks in favor of their statistically significantly higher efficacy in relation to the number of newly-formed axons (Figure 1), the degree of the presence of connective tissue (Figure 2), neovascularization (Figure 3), and the number of Schwann cells (Figure 4) relative to other methods.

Table 1

Comparative overview of patohistological characteristics of the damaged nerve regeneration six weeks after intervention

Pathohistological characteristics	Groups			
	I	II	III	IV
Number of newly created axons	22[a]	26.5[b]	86.5	87
Degree of presence of connective tissue	4[b]	3.5[b]	2	2
Degree of neovascularization	1[b]	1[b]	3	3
Number of Schwann cells	10[b]	12[b]	83.5	82

Description of the groups and histological methods are given in the paragraph Methods.
Statistical significance: [a]$p < 0.05$ vs. Groups II, III, and IV (Kruskal-Wallis and Mann-Whitney-Wilcoxon test); [b]$p < 0.05$ vs. Groups III and IV (Kruskal-Wallis and Mann-Whitney-Wilcoxon test).

Table 2

Comparative overview of pathophysiological characteristics of the damaged nerve regeneration 10 weeks after intervention

Pathohistological characteristics	Groups			
	I	II	III	IV
Number of newly created axons	22[a]	26.5[b]	88	89
Degree of presence of connective tissue	3.5[b]	3.5[b]	2	1.5
Degree of neovascularization	1[b]	1[b]	3	3
Number of Schwann cells	9.5[b]	12[b]	84	84

Description of the groups and histological methods are given in the paragraph Methods.
Statistical significance: [a]$p < 0.05$ vs. Groups II, III, and IV (Kruskal-Wallis and Mann-Whitney-Wilcoxon test); [b]$p < 0.05$ vs. Groups III and IV (Kruskal-Wallis and Mann-Whitney-Wilcoxon test).

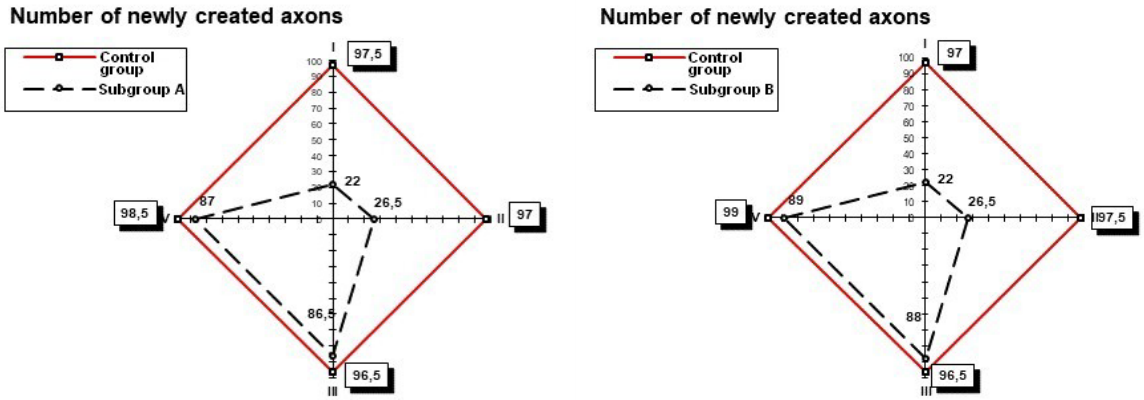

Fig. 1 − Comparison of the effects of the applied methods and results of the control group six weeks after intervention (subgroup A) and ten weeks after intervention (subgroup B) in terms of the number of newly created axons.
Description of the groups and histological methods are given in the paragraph Methods.

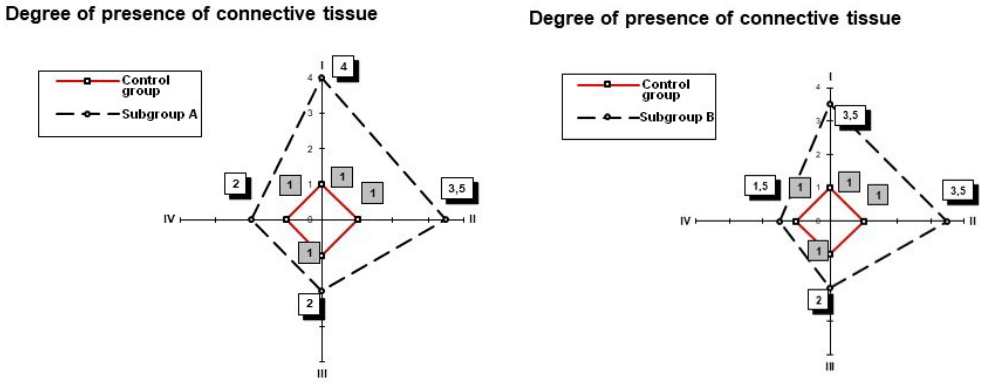

Fig. 2 − Comparison of the effects of the applied methods and results of the control group six weeks after intervention (subgroup A) and ten weeks after intervention (subgroup B) in terms of the degree of binding of connective tissue.
Description of the groups and histological methods are given in the paragraph Methods.

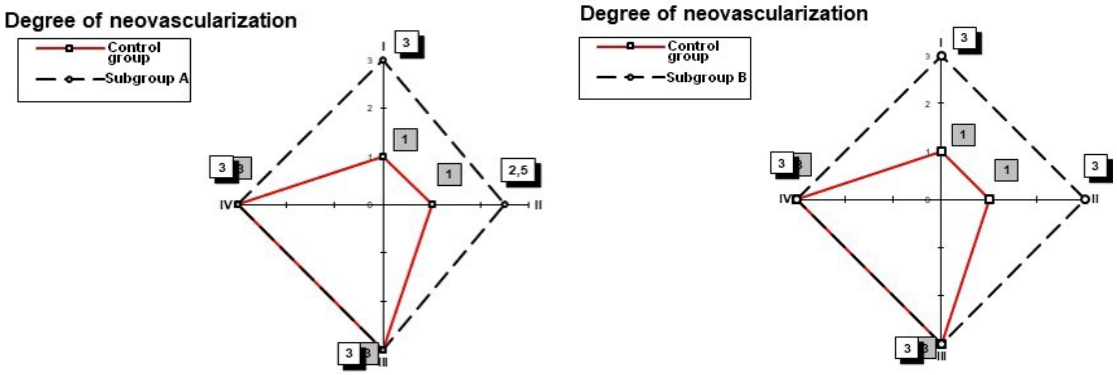

Fig. 3 − Comparison of the effects of the applied methods and results of the control group six weeks after intervention (subgroup A) and ten weeks after intervention (subgroup B) in terms of the degree of neovascularization.
Description of the groups and histological methods are given in the paragraph Methods.

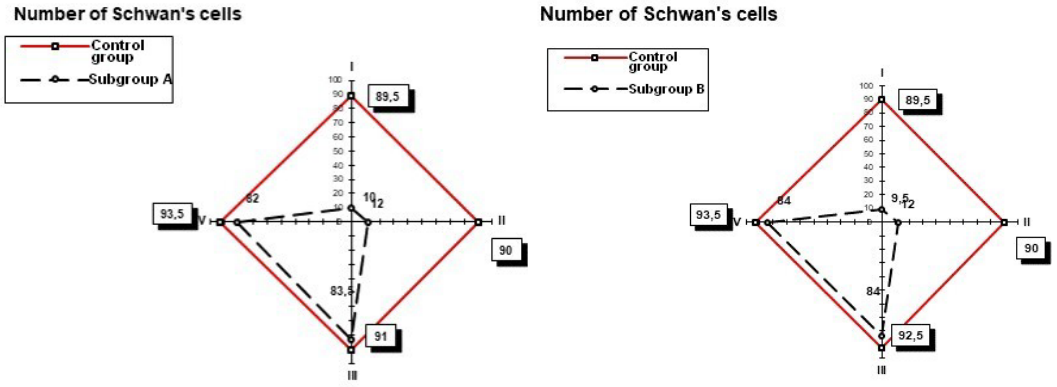

Fig. 4 − Comparison of the effects of the applied methods and results of the control group six weeks after intervention (subgroup A) and ten weeks after intervention (subgroup B) in terms of the number of Schwann cells.
Description of the groups and histological methods are given in the paragraph Methods.

Discussion

Injuries of peripheral nerves always lead to incomplete recovery due to the formation of connective tissue that creates a mechanical barrier in the process of axonal regeneration. Parts of the injured nerve due to scar healing lead to adhesion to the surrounding soft tissue. Despite the surgical methods of treatment of peripheral nerve injury, they lead to reduced functionality and prolonged recovery. Reduction of epineural degeneration and perineural scarring by using bio-

active factors that regulate connective proliferation and stimulating axonal regeneration on the distal portions of the injured nerve could lead to a rapid and complete recovery of the peripheral nerve [15].

Growth factors in the PRP are a class of natural biological mediators with local and systemic effects that regulate cell migration, coupling, and proliferation and also provide the accumulation of an extra cell matrix by binding to specific cell surface receptors [15].

The effect of PRP in the process of nerve regeneration in wounded nerves is preserved for now only within experimental studies, which showed different results in recent years and opened a new chapter in the polemics of the effectiveness of the PRP application [15].

Based on the published works from 2010 forward, primarily on experimental models for the regeneration of peripheral nerves with a special emphasis on the motor sciatic nerve and the results obtained on that occasion, it can be concluded that the application of PRP in the regeneration of peripheral nerves could be applied in clinical practice [16, 17].

The model of the study was the dominant sciatic nerve of rats and rabbits, after a section of the nerve, according to standardized protocols. Reconstruction was done through a suture or allograft in combination with PRP [16,18,19]. After a certain period, electromyographic examinations were performed, followed by histological analysis of the prepared part of the nerve. All results preferred the use of PRP in relation to control groups where it was not used [20, 21].

When it comes to degenerative changes, there are clinical studies based on the direct injection of PRP into the part of the damaged nerve [22, 23].

Favorable results were obtained after the application of PRP in patients with carpal syndrome, where electromyography tests were almost identical to animal model tests [24].

In the case of injuries of joint cartilage and muscle tendon, the PRP found a wide application in sports medicine with beneficial effects in the process of regeneration of cartilage hyaline with an improvement of function and slowing degenerative processes [23–25].

Platelet-rich plasma combined with bone substitutes or bone autografts can be used in jaw bone reconstruction in experimental and clinical studies [26, 27].

After a series of experimental studies of the effect of PRP on bone regeneration, clinical studies have confirmed the justification of the application of this method, especially when it comes to bone defects after fracture, in alveolar ridge enlargement, sinus elevation for the placement of dental implants after surgery of mandibular and maxillar cysts or tumors, where the resection of the jaws is necessary [26–30].

A large meta-analysis covering the period ending with 2014, with published papers in the field of clinical application of PRP in the bone regeneration of jawbone defects after tooth extraction (199 teeth in 156 patients), shows a positive effect of the PRP application. However, as this sample is still small for definitive conclusions, the results must be carefully interpreted with a proposal to work on a better standardization of experimental design in order to better understand the effects of the effectiveness of the PRP application [31].

The primary aim of our study was to give a contribution in terms of a more effective understanding of the use of PRP in the process of nerve regeneration.

Based on the statistical parameters, we concluded that regardless of the time interval (six and ten weeks), there is no difference in relation to the parameters tested.

The number of axons, Schwann cells, and newly created blood vessels are significantly dominant in Groups III and IV, which explains that the FG we used in Group IV together with PRP has no function in the process of nerve regeneration but is rather used as a tissue adhesive whose properties are proven much earlier.

Groups III and IV, in which PRP is used, have almost identical results, which supports the effectiveness of using this biological agent.

When it comes to the degree of connective tissue formation, the results indicate the smallest percentage of representation in Groups III and IV (a lower value indicates better results based on the graded gradation).

Based on all the results, we concluded that there is no statistical significance in relation to the period of the regeneration process. We also concluded that a six-week period could represent the optimum time in the process of nerve regeneration, as the examined values did not significantly change ten weeks after the reconstruction of the nerve was performed.

Conclusion

Our results suggest that using PRP promotes facial nerve regeneration in an animal model of facial nerve axotomy. Laboratory parameters indicated that the platelet count in the PRP was up to four times higher than in peripheral blood, which means that the growth factor concentration was significantly higher in PRP, which can explain its efficiency in the healing process. Based on the obtained histological analyses, we noticed that six weeks after the application of PRP in the process of reconstruction of the facial nerve in experimental animals, the number of Schwann cells and newly formed axons was approximately the same as in the healthy nerve (over 95% success rate). The degree of neovascularisation is identical to that of a healthy nerve, suggesting the efficacy of VEGF from the applied PRP. The connective tissue is present in traces, and knowing that the healing process greatly inhibits the scarring connective tissue, this parameter undoubtedly confirms the effectiveness of this method due to the increased concentration of growth factors that prevent the process of creating the scar tissue. Bearing in mind all these parameters, we can suggest that the PRP enters the standard surgical procedure in the process of regeneration of damaged facial nerves regardless of the type of injuries and under what circumstances it was created, with the recommendation that further experimental studies support the clinical application of PRP.

REFERENCES

1. *Sunderland S*. The anatomy and physiology of nerve injury. Muscle Nerve 1990; 13(9): 771–84.
2. *Jović N*. Facial paralysis: etiology, diagnosis and treatment. Belgrade: Vojnomedicinska Akademija; 2004. (Serbian)
3. *Klintworth* N, *Zenk J, Koch M, Iro H*. Postoperative complications after extracapsular dissection of benign parotid lesions with particular reference to facial nerve function. Laryngoscope 2010; 120(3): 484–90.
4. *Asma A, Marina MB, Mazita A, Fadzilah I, Mazlina S, Saim L*. Iatrogenic facial nerve palsy: lessons to learn. Singapore Med J 2009; 50(12): 1154–7.
5. *Davis RE, Telischi FF*. Traumatic facial nerve injuries: review of diagnosis and treatment. J Craniomaxillofac Trauma 1995; 1(3): 30–41.
6. *Gordin EA, Daniero JJ, Krein H, Boon MS*. Parotid gland trauma. Facial Plast Surg 2010; 26(6): 504–10.
7. *Gierek T, Majzel K, Slaska-Kaspera A, Gwóźdź-Jezierska M*. The results of surgical treatment of the facial nerve paralysis. Otolaryngol Pol 2007; 61(4): 383–6. (Polish)
8. *Gavron JP, Clemis JD*. Hypoglossal-facial nerve anastomosis: a review of forty cases caused by facial nerve injuries in the posterior fossa. Laryngoscope 1984; 94(11): 1447–50.
9. *Conley J, Baker DC*. Hypoglossal-facial nerve anastomosis for reinnervation of the paralyzed face. Plast Reconstr Surg 1979; 63(1): 63–72.
10. *Messora MR, Hitomi Nagata MJ, Chaves Furlaneto FA, Menegati Dornelles RC, Mogami Bomfim SR, Tatiana Miranda Deliberador,* et al. A standardized research protocol for platelet-rich plasma (PRP) preparation in rats. RSBO 2011; 8(3): 299–304.
11. *Ornelas L, Padilla L, Di Silvio M, Schalch P, Esperante S, Infante RL*, et al. Fibrin glue: an alternative technique for nerve coaptation--Part II. Nerve regeneration and histomorphometric assessment. J Reconstr Microsurg 2006; 22(2): 123–8.
12. *Izquierdo O, Alvarez R, Aparicio P, Castellanos J, Dominguez E*. Effect of platelet-rich plasma on peripheral nerve regeneration, model in rat. J Bone Joint Surg 2011; 93: 132–3.
13. *Küçük L, Günay H, Erbaş O, Küçük Ü, Atamaz F, Coşkunol E*. Effects of platelet-rich plasma on nerve regeneration in a rat model. Acta Orthop Traumatol Turc 2014; 48(4): 449–54.
14. *Duka M, Lazić Z, Bubalo M*. Effect of local administration of platelet-rich plasma and guided tissue regeneration on the level of bone resorption in early dental implant insertion Vojnosanit Pregl 2008; 65(6): 462–8. (Serbian)
15. *Giorgetti M, Siciliano G*. Platelet-rich plasma: the role in neural repair. Neural Regen Res 2015; 10(12): 1920–1.
16. *Lichtenfels M, Colomé L, Sebben AD, Braga-Silva J*. Effect of Platelet Rich Plasma and Platelet Rich Fibrin on sciatic nerve regeneration in a rat model. Microsurgery 2013; 33(5): 383–90.
17. *Abbasipour-Dalivand S, Mohammadi R, Mohammadi V*. Effects of Local Administration of Platelet Rich Plasma on Functional Recovery after Bridging Sciatic Nerve Defect Using Silicone Rubber Chamber; An Experimental Study. Bull Emerg Trauma 2015; 3(1): 1–7.
18. *Zheng C, Zhu Q, Liu X, Huang X, He C, Jiang L*, Q, et al. Improved peripheral nerve regeneration using acellular nerve allografts loaded with platelet-rich plasma. Tissue Eng Part A 2014; 20(23–24): 3228–40.
19. *Fatemi MJ, Pakfetrat F, Akhoondinasab MRA, Mansouri K, Moosavi SJ, Hosseini SA*, et al. The effect of sub-epineural platelet-rich plasma (PRP) on regeneration of the sciatic nerve in a rat model. Tehran Univ Med J 2016; 73(10): 693–9.
20. *Giannessi E, Coli A, Stornelli MR, Miragliotta V, Pirone A, Lenzi C*, et al. An autologously generated platelet-rich plasma suturable membrane may enhance peripheral nerve regeneration after neurorraphy in an acute injury model of sciatic nerve neurotmesis. J Reconstr Microsurg 2014; 30(9): 617–26.
21. *Sabongi RG, De Rizzo LA, Fernandes M, Valente SG, Gomes dos Santos JB, Faloppa F*, et al. Nerve regeneration: is there an alternative to nervous graft? J Reconstr Microsurg 2014; 30(9): 607–16.
22. *Sánchez M, Anitua E, Delgado D, Sanchez P, Prado R, Goiriena JJ,* et al. A new strategy to tackle severe knee osteoarthritis: Combination of intra-articular and intraosseous injections of Platelet Rich Plasma. Expert Opin Biol Ther 2016; 16(5): 627–43.
23. *Filardo G, Kon E, Pereira Ruiz MT, Vaccaro F, Guitaldi R, Di Martino A,* et al. Platelet-rich plasma intra-articular injections for cartilage degeneration and osteoarthritis: single- versus double-spinning approach. Knee Surg Sports Traumatol Arthrosc 2012; 20(10): 2082–91.
24. *Filardo G, Kon E, Roffi A, Di Matteo B, Merli ML, Marcacci M*. Platelet-rich plasma: why intra-articular? A systematic review of preclinical studies and clinical evidence on PRP for joint degeneration. Knee Surg Sports Traumatol Arthrosc 2015; 23(9): 2459–74.
25. *de Almeida AM, Demange MK, Sobrado MF, Rodrigues MB, Pedrinelli A, Hernandez AJ*. Patellar tendon healing with platelet-rich plasma: a prospective randomized controlled trial. Am J Sports Med 2012; 40(6): 1282–8.
26. *Taschieri S, Testori T, Corbella S, Weinstein R, Francetti L, Di Giancamillo A,* et al. Platelet-Rich Plasma and Deproteinized Bovine Bone Matrix in Maxillary Sinus Lift Surgery: A Split-Mouth Histomorphometric Evaluation. Implant Dent 2015; 24(5): 592–7.
27. *Kumar KA, Rao JB, Pavan Kumar B, Mohan AP, Patil K, Parimala K*. A prospective study involving the use of platelet rich plasma in enhancing the uptake of bone grafts in the oral and maxillofacial region. J Maxillofac Oral Surg 2013; 12(4): 387–94.
28. *Eskan MA, Greenwell H, Hill M, Morton D, Vidal R, Shumway B,* et al. Platelet-rich plasma-assisted guided bone regeneration for ridge augmentation: a randomized, controlled clinical trial. J Periodontol 2014; 85(5): 661–8.
29. *Daif ET*. Effect of autologous platelet-rich plasma on bone regeneration in mandibular fractures. Dent Traumatol 2013; 29(5): 399–403.
30. *Menezes DJ, Shibli JA, Gehrke SA, Beder AM, Sendyk WR*. Effect of platelet-rich plasma in alveolar distraction osteogenesis: a controlled clinical trial. Br J Oral Maxillofac Surg 2016; 54(1): 83–7.
31. *Del Fabbro M, Corbella S, Taschieri S, Francetti L, Weinstein R*. Autologous platelet concentrate for post-extraction socket healing: a systematic review. Eur J Oral Implantol 2014; 7(4): 333–44.

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CASE REPORTS

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Large schwannoma of the median nerve at the distal forearm

Veliki švanom medijalnog nerva u distalnom delu podlaktice

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Abstract

Introduction. Schwannoma, also known as neurilemmoma, is a rare but one of the most common tumors of the peripheral nerves. It originates from Schwann cells of the peripheral nerve sheaths. Schwannoma mostly occurs in adults between 20 to 70 years of age. The regions where the tumor most commonly occurs are the head and neck, but it can occur almost anywhere in the body or the organs. Schwannomas are usually up to 2.5 cm in size but may grow to 4–5 cm. In this study, the rare case of large schwannoma of the median nerve in the distal part of the forearm is presented. **Case report.** A 46-year-old male patient was referred to a plastic surgeon with a diagnosis of lipoma on the anterior side of the distal third of the left forearm. Ultrasound and magnetic resonance imaging were done, and the surgery was performed after that. An encapsulated tumor of the median nerve was found, and the tumor was completely removed without nerve damage. Histological analysis showed a benign schwannoma of cellular type and biphasic shape. In the postoperative course, there was transient paresthesia. One year after surgery, no tumor recurrence nor neurological deficit was recorded. **Conclusion.** Schwannoma is the most common benign tumor of peripheral nerves. Schwannomas over 5 cm in size are extremely rare. Appropriate physical examination, preoperative imaging studies, and histological verification are required for the final diagnosis. The method of choice in treating large schwannomas is complete surgical excision.

Key words:
diagnosis; median nerve; neurilemmoma; peripheral nervous system neoplasms; schwann cells.

Apstrakt

Uvod. Švanom, poznat i kao neurilemom, je redak ali jedan od najčešćih tumora perifernih nerava. Potiče od Švanovih ćelija nervnog omotača. Švanom se najčešće javlja kod odraslih osoba, između 20 i 70 godina starosti. Uglavnom se javlja na glavi i vratu, ali se može pojaviti na skoro svim delovima tela ili u organima. Švanomi su najčešće veličine do 2,5 cm, ali mogu dostići i dimenzije do 4–5 cm. U ovom radu prikazan je bolesnik sa švanomom medijalnog nerva u distalnom delu podlaktice. **Prikaz bolesnika.** Osoba muškog pola, starosti 46 godina, upućena je specijalisti platične hirurgije, sa dijagnozom lipoma na prednjoj strani distalne trećine leve podlaktice. Urađeni su ultrasonografija i magnetska rezonanca, a nakon toga je urađena operacija. Utvrđeno je da se radi o inkapsuliranom tumoru medijalnog nerva i tumor je uklonjen u celini, bez oštećenja nerva. Histološka analiza je pokazala da se radilo o benignom švanomu celularnog tipa i bifaznog oblika. U postoperativnom toku postojala je prolazna parestezija. Godinu dana nakon operacije nije zabeležen recidiv tumora, niti neurološki deficit. **Zaključak.** Švanom je najčešći benigni tumor perifernih nerava. Švanomi dimenzija preko 5 cm su izuzetno retki. Adekvatan klinički pregled, preoperativna dijagnostika i histološka verifikacija neophodni su za postavljanje konačne dijagnoze. Kompletna hirurška ekscizija velikih švanoma je metoda izbora u lečenju ovih tumora.

Ključne reči:
dijagnoza; n. medianus; švanom; nervni sistem, periferni, neoplazme; švanove ćelije.

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Introduction

Schwannoma (neurinoma, neurilemmoma) is a tumor of nervous tissue that arises from Schwann cells of the nerve sheath. It is almost always a benign tumor (in more than 90% of cases), and with neurofibroma, it is the most common tumor of nervous tissue [1]. Schwannoma accounts for less than 8% of soft tissue neoplasms. It usually occurs in middle age, equally regarding race and gender [2]. It can develop almost anywhere in the body, but the most common localizations are the head and neck (more than 40% of cases). The vestibular nerve is the most common location, with a prevalence of 1 in 2,000 adults. Incidentally diagnosed schwannoma on magnetic resonance imaging (MRI) study or autopsy are more common. The occurrence of schwannoma in parenchymatous organs is rare.

Schwannoma is oval, soft, light in color, encapsulated and clearly demarcated, mobile, and usually up to 3 cm in diameter. Its growth is mostly slow and asymptomatic because it dislocates nerve fibers. In most cases, schwannoma is a solitary tumor, and multiple forms are rare and hereditary [1, 3]. Schwannoma is thought to be caused by dysfunction of the *Nf2* tumor suppressor gene. The histological appearance of schwannoma is biphasic because it consists of the Antoni A and Antoni B areas. Antoni A areas are composed of compact spindle cells with elongated nuclei arranged in fascicles and bands. Antoni B areas consist of hypocellular zones and a loose myxoid matrix. There are several forms of schwannoma: classical (most common), cellular, plexiform, and melanotic [4]. The diagnosis of schwannoma is based on the clinical picture, ultrasound examination, MRI, and histological findings. The differential diagnosis includes various subcutaneous soft tissue tumors. The treatment is surgical, and it involves complete surgical excision of the entire tumor, with generally good results. Tumors of the forearm soft tissues are numerous because of the different tissues in that region. Many of them, like schwannoma, are oval, soft, mobile, and painless. Besides, forearm nerve tumors are rare, and they can be misdiagnosed as fibroma, lipoma, fibrolipoma, ganglion, giant cell tumors of tendon sheaths, subcutaneous hemangiomas, malignant subcutaneous sarcomas, etc. There are not many reports of cases of larger median nerve schwannomas in the forearm [5].

Therefore, the aim of this study was to present a rare case of the large schwannoma of the median nerve in the distal part of the forearm, the diagnostic procedures, and the treatment result.

Case report

A 46-year-old man was referred to a plastic surgeon with a diagnosis of a lipoma of the left forearm. Six months earlier, the patient noticed a subcutaneous growth that gradually increased. Pain occurred occasionally, especially during flexion of the fingers and after pressure on the tumor. The tumor was on the anterior side of the distal third of the left forearm, as a solitary subcutaneous soft and mobile oval mass, with well-defined borders (Figure 1). The dimensions of the tumor were about 6 × 4 cm, and there were no changes on the skin above the tumor. The tumor was mobile from medial to lateral but not in the longitudinal direction. There were no pulsations nor fluctuations, and Tinel's sign was positive. The neurocirculatory finding on the hand was normal, and the axillary lymph glands were not enlarged. Additional tests, such as ultrasound examination and MRI were performed. Ultrasonography showed a round, homogeneous, and hypoechoic tumor with regular margins and no internal blood flow (Figure 2). MRI showed a well-circumscribed mass in the distal part of the anterior forearm with the displacement of surrounding structures and without direct invasion. Typical MRI features for schwannoma were present – T1-weighted iso-to hypointensity (Figure 3) and T2-weighted heterogeneous hyperintensity (Figure 4).

Surgery was performed under general anesthesia and under tourniquet control. After a longitudinal lazy-S incision, an encapsulated tumor of the median nerve was identified

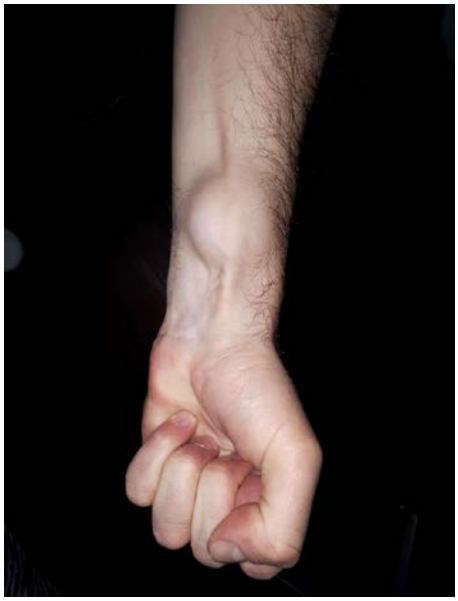

Fig. 1 – Soft tissue mass of the distal forearm.

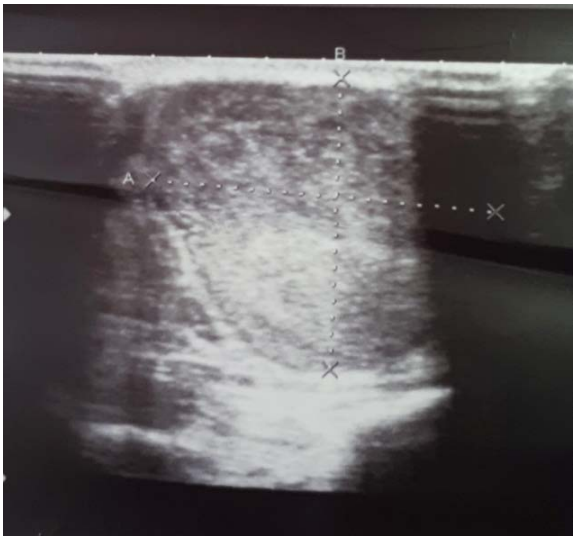

Fig. 2 – Ultrasonography finding of a round, homogeneous, and hypoechoic tumor, with regular margins and no internal blood flow.

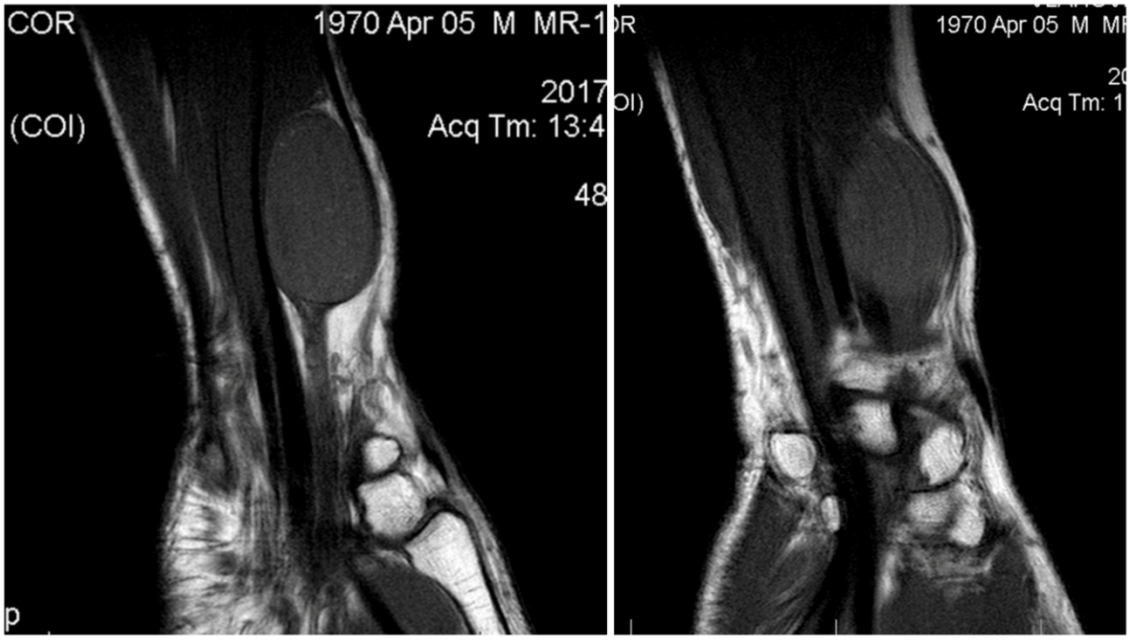

Fig. 3 – T1-weighted sagittal magnetic resonance images of a well-defined large oval mass at the flexor aspect of the forearm along the course of the median nerve, proximal to the carpal tunnel.

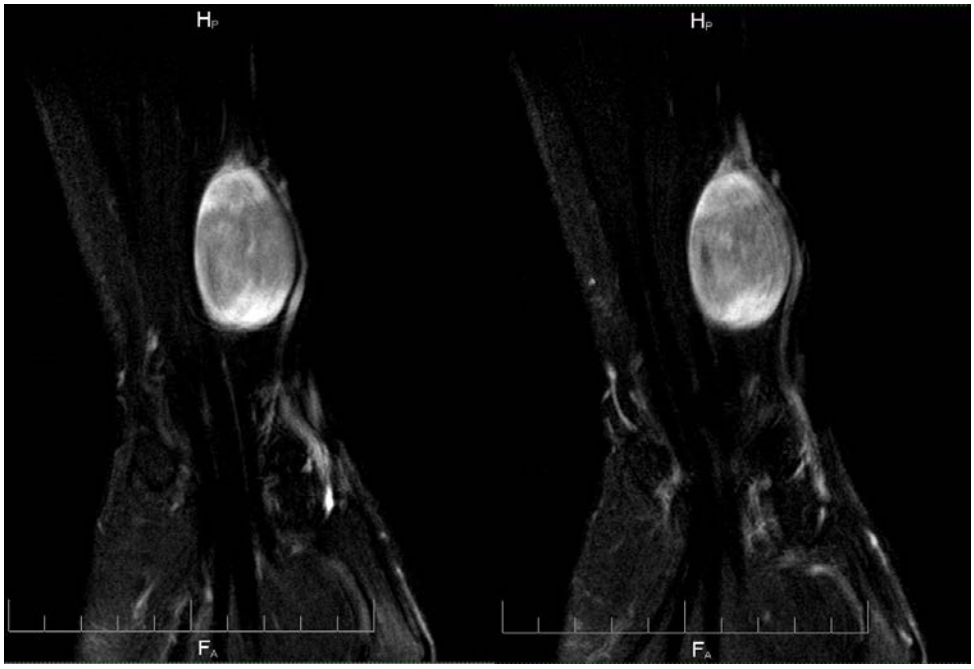

Fig. 4 – T2-weighted sagittal magnetic resonance images that show heterogeneous hyperintensity of well-defined large oval mass at the flexor aspect of the forearm.

(Figure 5). The color of the tumor was yellow, and it was very similar to lipoma. The tumor was exposed using binocular loupes, and after identifying the site where there was a splaying of fascicles, an epineurotomy was done longitudinally. Normal nerve fibers were identified proximally, and the tumor was dissected from fascicles and shelled out from the median nerve. The nerve was inspected to confirm continuity. The dimensions of the tumor were 6.2 × 4.3 cm.

Histological examination showed a well-encapsulated cellular proliferation composed of a biphasic tumor with highly cellular areas, called Antoni A area (Figure 6a), nuclear palisades area (Verocay bodies, Figure 6b), in addition to hypocellular areas (Antoni B, Figure 6c). On immunohistochemistry, the tumor cells were strongly positive for S-100 (Figure 6d).

In the postoperative period, there was transient paresthesia in the innervation area of the median nerve, and the wound healed regularly with good motor function (Figure 7). One year after surgery, there were no signs of tumor recurrence or neurological deficit. The ninhydrin test confirmed a good sensory function.

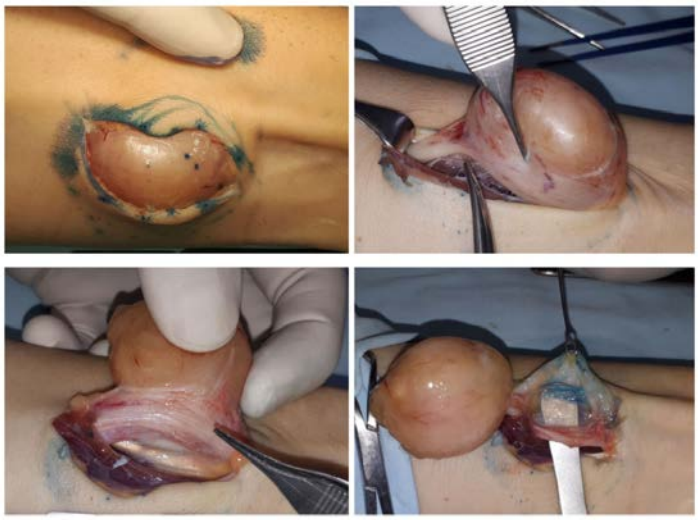

Fig. 5 – A large tumor of the median nerve, during surgery.

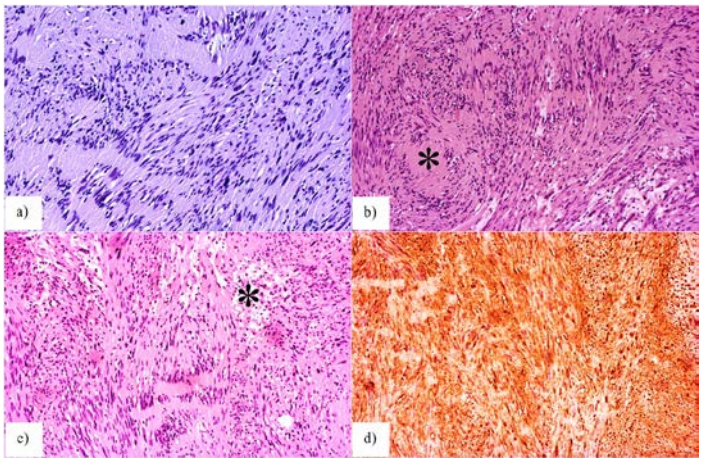

Fig. 6 – Histological examination of the tumor, hematoxylin and eosin staining, × 400 magnification: a) Tumor cells arranged in Antoni A areas; b) Nuclear palisading around the fibrillary process (Verocay body, marked by an asterisk); c) Tumor composed of loose hypocellular areas (Antoni B area, marked by an asterisk); d) Immunohistochemical staining of the tumor cells with S-100 showing strong immunoreactivity of the tumor.

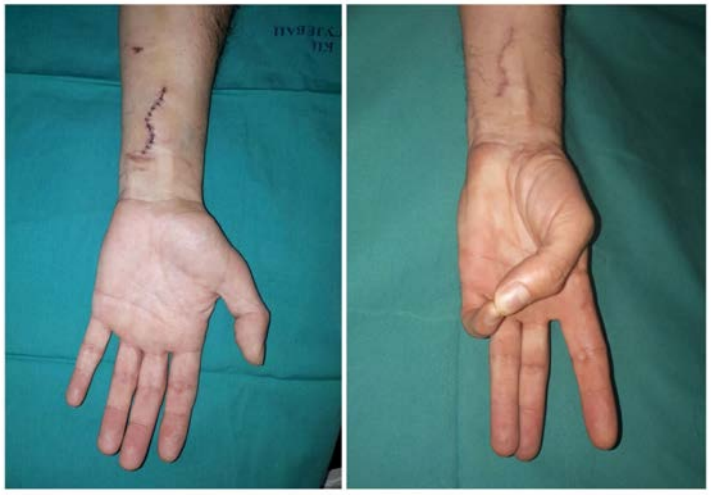

Fig. 7 – Seven days (left side image) and three months (right side image) after the surgery with good wound healing and good hand function.

Discussion

Schwannoma is a rare and the most common nerve tumor that can affect central and peripheral nerves of all types (motor, sensitive, and autonomic). It belongs to the group of peripheral nerve sheath tumors (PNST) [4, 6, 7]. Schwannoma was reported first by Verocay in 1910 and was named "schwannoma" by Masson in 1932. Schwannoma is usually benign, solitary, and asymptomatic [3]. Schwannomas almost always appear in adults. They are extremely rare in children, and only a few cases of congenital schwannoma have been described. Schwannoma most commonly occurs in adults between 20 and 50 years of age, with no difference regarding gender [8]. The presented patient was 46 years old when schwannoma was diagnosed. Almost half of the schwannomas are located on the head, mostly the vestibular nerve, but they can be found in any part of the body, even in the bones [9]. Most schwannomas are solitary, with diameters from 1.5 to 3 cm [10]. The schwannoma of the presented patient was unusually large, with dimensions of 6 × 4 cm. A noticeable subcutaneous mass, which is oval and soft, may be seen on the extremities. The tumor may present in different colors, such as yellow, white, brown, gray, or pink.

Symptomatology depends on the nerve type, tumor size, and anatomical region. Most schwannomas are asymptomatic. Numbness and pain occur in larger schwannomas and in those located in specific areas [11]. Schwannomas are usually painful to palpation on and around the mass when located superficially on extremities. The main difference between schwannoma and neurofibroma is that neurofibroma infiltrates the nerve. Lipoma is often found on the forearm as a soft, painless mass, mobile in all directions, even vertically, and it is usually not fixed in depth. They are similar to fibrolipomas but are of a slightly harder consistency. Ganglions are mostly painless and soft in consistency but often fixed to deeper structures, such as tendon sheaths, and are uncommon in the forearm. Giant cell tumors of tendon sheaths are lobular, are not soft, are fixed to tendons, and are not common tumors in the forearm. Subcutaneous hemangiomas can be found as soft and hard tumors, usually have a different color from the surrounding skin, can be pulsating, and sometimes have a lobular surface. Malignant subcutaneous sarcomas are less common, usually have a harder consistency, are fixed, and often cause stronger motor and sensory deficits.

Schwannoma can be associated with genetic disorders. The occurrence of multiple schwannomas is referred to as schwannomatosis, which is an autosomal dominant inherited disease. It is also called neurofibromatosis (NF) type 3, and there is a mutation in *SMARCB1* or *INI1* tumor suppressor gene [11].

Malignant schwannoma is rare and belongs to the group of sarcomas referred to as malignant PNST. It is most common in type I NF (gene defect in chromosome 17), with a risk of 10%. Malignant alteration of schwannoma to epithelioid sarcoma has also been described [4].

Subcutaneous tumors of the forearm are relatively common, but tumors of the forearm nerves are rare. There are some statistics on the frequency of subcutaneous soft tissue tumors. Still, in the hand region, the most common are ganglia (more than 50% of cases), followed by giant cell tumors of tendon sheaths, mucous cysts, lipomas, glomus tumors, vascular tumors, and, finally, tumors of the nerves (schwannoma, neurofibroma) [12, 13]. Concerning hands, schwannomas of the median nerve make up 0.1 to 0.3% of all hand tumors. In the presented patient, schwannoma of the median nerve in the distal part of the forearm had an extremely rare localization for this tumor type.

The diagnosis of schwannoma is made by physical examination, additional imaging, and biopsy [13]. On extremities, there is a soft and oval subcutaneous mobile mass throughout the nerve, typically without motor deficit. Differential diagnosis includes many soft tissue tumors. It is difficult to distinguish schwannoma from neurofibroma in a physical examination because, due to the slow growth of a benign nerve tumor, nerve function adapts to this compression effect [14]. Schwannomas can be distinguished from lipomas and ganglions by painful palpation and the Hoffman-Tinel sign. If there is a motor deficit, malignancy should be suspected. The significance of this case presentation is the fact that the patient was referred to a plastic surgeon by a primary care doctor with an initial diagnosis of a lipoma. That can be misleading for some less experienced surgeons who might believe that it really is a lipoma, and as a consequence, iatrogenic nerve injury may occur during the surgery.

Ultrasonography of schwannoma shows round or ovoid, hypoechogenic homogenous mass with clear margins [15]. On the extremities, this finding is similar to ganglia when there is a small lesion or to lipomas and fibrolipomas when the tumor is larger. In smaller schwannomas, there is a cystic appearance, but in large and long-lasting tumors, heterogeneous lesions may be seen, with signs of hemorrhage, calcifications, and fibrosis [16]. In that case, it is not easy to distinguish it from vascular lesions and even malignancies.

MRI is essential in diagnosing schwannoma. It is sometimes the diagnostic method of choice [16], especially for schwannomas of smaller nerves. MRI finding is typical, with T1-weighted iso-to hypointensity, hyperintensity on T2-weighted images, and postcontrast enhancement [12]. The borders of the tumor are smooth and well-defined, with a split fat sign. Otherwise, malignancy can be suspected, particularly if the tumor is over 5 cm in size. Entering and exiting nerve sign is usually seen on extremities. MRI is good for evaluating the anatomical site of the tumor, the size and extent of the tumor, and the relationships between the nerve and other tissues [17]. It is believed that MRI angiography has up to 91% accurate diagnosis of schwannoma.

The main treatment for schwannoma is surgical removal. Complete tumor excision is usually possible since the tumor does not involve nerve fibers. On the extremities, it is important to perform the surgery under tourniquet control, using the meticulous surgical technique to remove the tumor completely without damaging the nerve fibers. After dissection from proximal to distal and tumor identification, epineurotomy is performed, followed by circumferential dissection. In this way, the whole tumor is usually removed without any

difficulty [18]. In contrast, excision of neurofibroma and malignant schwannoma is often accompanied by nerve damage, and nerve grafting may be required. Complications after the excision of schwannoma are rare and transient paresthesia is the most common as a result of neuropraxia. Motor deficits are rare, and the recurrence rate is low. Early physical therapy is of great importance [9]. The patient we present in the paper had no complications after the surgery, and the hand function was preserved.

The limitation of this article was the fact that the MRI was done without contrast; therefore, we were unable to present the images with post-contrast T1-weighted sequences, which would have been better since they show more clear characteristics of schwannoma.

Conclusion

Median nerve schwannomas in the forearm are rare, especially those of larger dimensions. It is essential to distinguish them from other tumors of soft tissues, especially lipomas. The significance of this case study is that it presents a rare large-sized tumor of the median nerve with a rare localization. Physical examination is mandatory, but ultrasonography and MRI are very useful in choosing an appropriate surgical technique. Histopathological examination is also mandatory for differential and final diagnosis. Complete excision of large schwannomas in extremities is possible with adequate diagnosis and microsurgical technique under tourniquet control, without major complications or recurrence after surgery.

Acknowledgement

This study was partially financed by grant JP 12-18, "Histological and microbiological analysis of preserved human skin grafts", given by the Faculty of Medical Sciences, University of Kragujevac, Serbia, and by grant III 41007 given by the Serbian Ministry of Education, Science, and Technical Development.

REFERENCES

1. *Sonig A, Gandhi V, Nanda A.* From the cell of Schwann to schwannoma- a century's fruition. World Neurosurg 2014; 82(5): 906–11.
2. *Kehoe N, Reid R, Semple J.* Solitary benign peripheral-nerve tumours. Review of 32 years' experience. J Bone Joint Surg Br 1995; 77(3): 497–500.
3. *Rockwell G, Thoma A, Salama S.* Schwannoma of the hand and wrist. Plast Reconstr Surg 2003; 111(3): 1227–32.
4. *Kurtkaya-Yapicier O, Scheithauer B, Woodruff J.* The pathobiologic spectrum of Schwannomas. Histol Histopathol 2003; 18(3): 925–34.
5. *Knight DM, Birch R, Pringle J.* Benign solitary schwannomas: a review of 234 cases. J Bone Joint Surg Br 2007; 89(3): 382–7.
6. *Angelini A, Bevoni R, Biz C, Cerchiaro MC, Girolami M, Ruggieri P.* Schwannoma of the foot: Report of four cases and literature review. Acta Biomed 2019; 90(Suppl 1): 214–20.
7. *Hao X, Levine D, Yim J, Qi C, Firestone L, Beiser I,* et al. Schwannoma of foot and ankle: Seven case reports and literature review. Anticancer Res 2019; 39(9): 5185–94.
8. *Eroglu U, Yakar F, Zaimoglu M, Ozgural O, Kahilogulları G.* Median nerve schwannoma. ANZ J Surg 2019; 89(9): 1158–9.
9. *Salar M, Kaye MB.* Multiple Schwannomas of the Median Nerve: A Case Report and Review of the Literature. J Orth Case Reports 2020; 10(6): 60–3.
10. *Hakan T, Kılıç Y, Çelikoğlu E, Ekemen S.* An Unusual Schwannoma in the Proximal Forearm: A Case Report. Cureus 2019; 11(11): e6231.
11. *Barber CM, Fahrenkopf MP, Adams NS, Naum SC.* Multiple Peripheral Schwannomas. Eplasty 2018; 18: ic7.
12. *Unnithan AKA, Joseph A.* Schwannoma of median nerve: Case report and review of literature. Interdisciplin Neurosurg 2019; 17: 75–8.
13. *Hubert J, Landes G, Tardif M.* Schwannoma of the median nerve. J Plast Surg Hand Surg 2013; 47(1): 75–7.
14. *Padasali PS, Shankaregowda VS, Kshirsagar SD.* Median nerve schwannoma: A case and review of literature. Asian J Neurosurg 2015; 10(3): 212–5.
15. *Vlychou M, Dailiana ZH.* Ancient schwannoma of the hand. J Hand Surg Am 2011; 36(12): 2030–3.
16. *Rutka V, Castel T, Burnier M, Herzberg G.* Atypical schwannoma of the median nerve. A case report. Hand Surg Rehabil 2019; 38(4): 273–5.
17. *Boufettal M, Azouz M, Rhanim A, Abouzahir M, Mahfoud M, Bardouni AE,* et al. Schwannoma of the median nerve: diagnosis sometimes delayed. Clin Med Insights Case Rep 2014; 7: 71–3.
18. *Dusad T, Meena DS, Saini N, Sharma Y, Khurana D.* Schwannoma of the Median Nerve at Mid Forearm Level. J Orthop Case Rep 2016; 6(2): 66–8.

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CASE REPORT
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Li-Fraumeni syndrome – a case report

Li-Fraumenijev sindrom

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Abstract

Introduction. Li-Fraumeni syndrome (LFS) is a hereditary familial predisposition to a wide range of certain, often rare malignant diseases. Patients also have an increased risk of developing secondary and even tertiary malignancies throughout their lifetime. The most common malignancies are soft-tissue and bone sarcomas, breast cancer, brain tumors, adrenocortical carcinoma, and acute leukemia. The syndrome is inherited as an autosomal dominant disorder. In most families with LFS, germline mutations of the tumor protein have been identified on the *TP53* gene. To our knowledge, this is the second case report of LFS that has been reported in our country so far. **Case report.** We present five members of the same family with malignant diseases typical for LFS. A woman at the age of 21 had recurrent astrocytoma and mediastinal liposarcoma. Her older sister had rhabdomyosarcoma and liver cancer and died at the age of 18. The mother of their father was diagnosed with breast cancer at the age of 45, and she died at the age of 52. The father's sister had osteosarcoma and died before the age of 40. The father was diagnosed with lung adenocarcinoma at the age of 49, two years after the death of his second daughter. Genetic analysis identified a pathogenic, heterozygous germline mutation of the *TP53* gene. He also has a third, 8-year-old daughter for whom he denied testing for LFS. **Conclusion.** Genetic analysis for LFS of all family members is required in patients with rare and multiple malignancies but also frequent and early onset malignancies in the family. Screening for the detection of early cancer manifestation is the key to prolonged survival in people with LFS.

Key words:
diagnosis; family; genetic diseases, inborn; li-fraumeni syndrome; mutation; serbia.

Apstrakt

Uvod. Li-Fraumenijev sindrom (LFS) je nasledna porodična predispozicija za širok spektar određenih, često retkih malignih bolesti. Bolesnici, takođe, imaju povećan rizik od razvoja sekundarnih, pa čak i tercijarnih malignih bolesti tokom čitavog života. Najčešći su sarkomi mekih tkiva i kostiju, karcinom dojke, tumori mozga, adrenokortikalni karcinom i akutna leukemija. Sindrom se nasleđuje kao autozomno dominantni poremećaj. U većini porodica sa LFS, identifikovane su heterozigotne mutacije na genu *TP53*. Po našem saznanju, ovo je drugi prikaz LFS u Srbiji. **Prikaz bolesnika.** Prikazujemo porodicu u kojoj su kod pet članova dijagnostikovane maligne bolesti tipične za LFS. Žena u 21. godini života lečena je zbog rekurentnog astrocitoma i medijastinalnog liposarkoma. Njena starija sestra imala je rabdomiosarkom i karcinom jetre i umrla je u 18. godini. Majci njihovog oca dijagnostikovan je karcinom dojke u 45. godini, a umrla je u 52. godini života. Očeva sestra imala je osteosarkom i umrla je pre 40. godine života. Njihovom ocu dijagnostikovan je adenokarcinom pluća u životnom dobu od 49 godina, dve godine nakon smrti druge ćerke. Genetičkom analizom otkrivena je heterozigotna mutacija gena *TP53*. On takođe ima i treću, osmogodišnju ćerku za koju nije odobrio genetičko testiranje. **Zaključak.** Kod bolesnika sa retkim i višestrukim malignim bolestima, kao i kod čestih i ranih maligniteta u porodici, potrebna je genetička analiza za LFS svih članova porodice. Ciljana provera prisustva ranih manifestacija malignih bolesti ključna je za otkrivanje LFS i produženo preživljavanje obolelih od ovog sindroma.

Ključne reči:
dijagnoza; porodica; genetičke bolesti, urođene; li-fraumeni sindrom; mutacija; srbija.

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Introduction

Li-Fraumeni syndrome (LFS) is a hereditary familial predisposition to a wide range of certain, often rare malignant diseases. To date, approximately 400 families with LFS have been reported in the literature [1]. The prevalence is 1–9 : 100,000 inhabitants [2]. The syndrome was first recognized in 1969 by Frederick Li and Joseph Fraumeni Jr while studying pediatric and familial malignant tumors at the National Cancer Institute. They described multiple malignant diseases in children and young adults in four families and increased risk for multiple primary tumors [3]. LFS patients also have an increased risk of developing secondary and even tertiary malignancies throughout their lifetime. The most common are soft-tissue and bone sarcomas, breast cancer, brain tumors, adrenocortical carcinoma, and acute leukemia [4]. The lifetime risk of malignant disease in individuals with LFS is ≥ 70% for men and ≥ 90% for women [5]. This predisposition syndrome is inherited as an autosomal dominant disorder. In most families with LFS, germline mutations of tumor protein p53 gene (*TP53*) have been identified in chromosome 17p13.1, which codes for a transcription factor implicated in cell proliferation, apoptosis, and genomic stability [6].

A diagnosis of LFS and performance of the *TP53* gene mutation testing is considered for anyone with a personal and family history that meet one out of the following three criteria [1]: 1) if a person is diagnosed with a tumor from the LFS tumor spectrum, before the age of 46, which includes any of the following diseases – soft-tissue sarcoma, osteosarcoma, pre-menopausal breast cancer, brain tumor, adrenal cortical carcinoma, leukemia or lung cancer, and at least one first-degree or second-degree family member with an LFS-related tumor, except breast cancer which developed before the age of 56 or with multiple tumors; 2) if a person has multiple tumors, except multiple breast tumors, two of which belong to the LFS tumor spectrum and the first of which occurred before the age of 46; 3) if a person is diagnosed with adrenocortical carcinoma or a tumor in the choroid plexus, meaning a membrane around the brain, regardless of family history.

In addition, patients with anaplastic rhabdomyosarcoma, women with breast cancer prior to the age of 31, and patients with hypodiploid acute lymphoblastic leukemia and Sonic Hedgehog medulloblastoma should be tested, regardless of their family history [5,7].

Case report

Case 1

The proband was a woman at the age of 21. She was admitted in April 2016 to Neurology Department due to a headache that lasted for two months. She also reported occasional tingling in the left half of her face. She reported no history of loss of consciousness, convulsion, chronic diseases, or smoking. There was a family history of frequent malignancies. The mother of her father was diagnosed with breast cancer at the age of 45 and died at the age of 52. The father's sister was treated for osteosarcoma. She died before the age of 40. The proband's older sister had rhabdomyosarcoma and liver cancer and died at the age of 18. The family tree is shown in Figure 1. Case 1 is marked with an arrow.

Neurological examination found no significant discrepancies. The patient underwent diagnostic procedures. Blood analysis results, except for mild anemia, revealed normal findings. Electroencephalography found focal somatosensory seizures (Figure 2), and magnetic resonance imaging (MRI) showed an expansive change in the right parietal lobe of the brain (Figure 3).

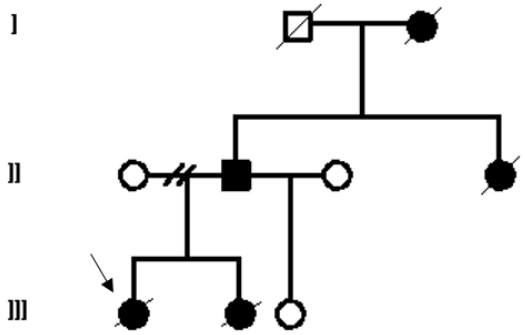

Fig. 1 – Family tree illustrating the presence of different malignant diseases in three generations. In the first generation, the father's mother had breast cancer and died at the age of 52. In the second generation, the father had lung adenocarcinoma; his sister had osteosarcoma and died at the age of 40. The third generation represents two daughters from the first marriage who had rhabdomyosarcoma, liver cancer, and astrocytoma and a third 8-year-old daughter from the second marriage, currently without cancer. Symbol of square – male; symbol of circle – female; affected person symbols are colored black; married or in partnership person symbols are connected with a horizontal line; divorced or separated person symbols are connected with a crossed horizontal line.

We concluded that the change in the parietal right lobe showed the characteristics of the expansive process. Neurosurgical treatment and histopathological (HP) verification of the change were performed. Astrocytoma anaplasticum (World Health Organisation – WHO, grade III) was diagnosed. Radiotherapy was performed in the optimal period, and the patient was controlled periodically until 2018. In April 2018, chest pain occurred, and a large mass in the upper mediastinum involving the superior vena cava was found on computed tomography (CT) (Figure 4). A surgical biopsy was performed, and the HP finding confirmed pleomorphic liposarcoma. A positron emission tomography can showed the dissemination of the tumor in the liver, pericardium, and skeletal system. Chemotherapy treatment was recommended. In June 2018, the patient was urgently admitted to the hospital due to severe headaches and signs of increased intracranial pressure. CT showed a recurrence of the brain tumor in the right parietal lobe. There were suspicious signs of bleeding in the tumor (Figure 5). An emergency craniotomy was performed. The

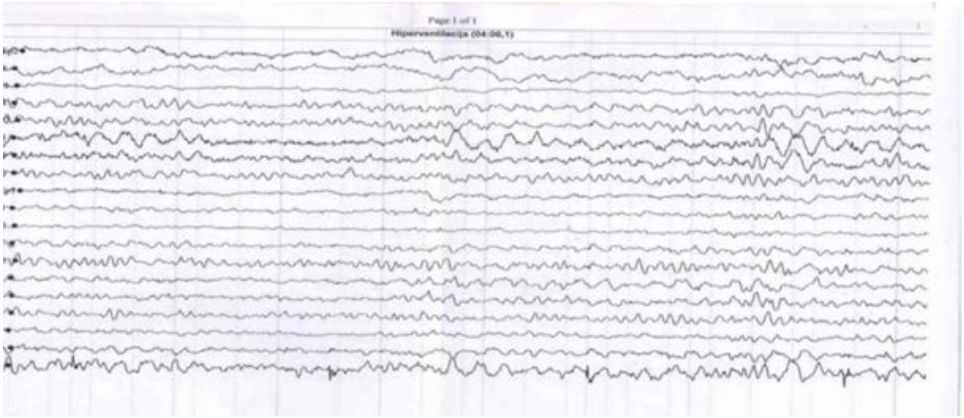

Fig. 2 – Electroencephalography finding shows focal somatosensory seizures.

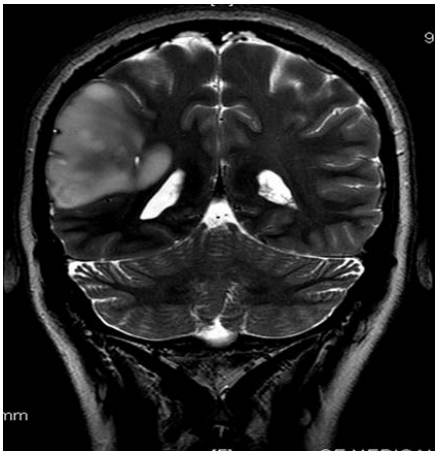

Fig. 3 – Head magnetic resonance imaging shows a tumor in the right parietal lobe of the brain.

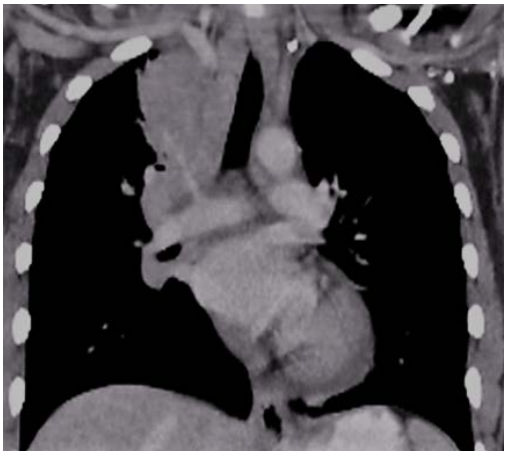

Fig. 4 – Chest computed tomography scan shows a tumor of the upper mediastinum involving the *superior vena cava.*

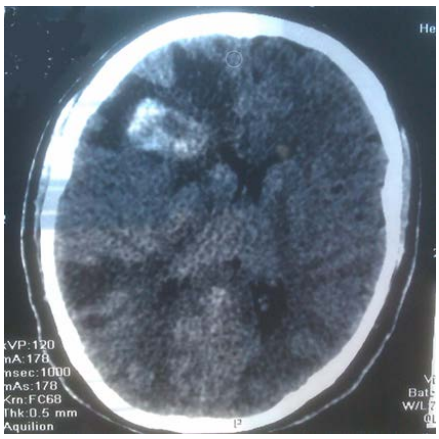

Fig. 5 – Head computed tomography scan shows recurrence of a brain tumor in the right parietal lobe with suspected bleeding.

tumor was extirpated and made external drainage of cerebrospinal fluid. HP finding confirmed recurrence of astrocytoma.

In the further course, there was no improvement in the patient's condition. The fatal outcome occurred in July 2018 at the age of 23.

Due to the existence of several rare malignant diseases in the family (grandmother, sister, and aunt), the LFS was suspected. The woman's father has been invited to genetic counseling several times but did not respond.

Case 2

The second case is the father of the first presented patient (Case 1). A man at the age of 49 was admitted to the Pulmonology Department due to cough and back pain in October 2020. In his history, he denied chronic diseases and smoking. His mother, two daughters (18 and 23 years), and a sister died of rare and multiple malignancies. He has a healthy 8-year-old daughter. Radiography and chest CT revealed a tumor in the left upper lung lobe, approximately 4 cm in diameter, with pleural effusion (Figures 6 and 7).

Due to back pain, radiography and skeletal scintigraphy were performed. Metastases were found in the spinal column. The bronchoscopy findings were normal. Pulmonary adenocarcinoma was confirmed from a transbronchial lung biopsy. Additional testing found epidermal growth factor receptor – EGFR mutation in exon 21 (L858R). The clinical stage was T2aN2M1c (bone metastases – OSs) – stage IVB. The oral administration of tyrosine kinase inhibitor – afatinib was started in November 2020. Palliative radiotherapy for metastatic disease in the spine was also applied in December 2020. On the last assessment in October 2021, there was a partial response according to Response Evaluation Criteria In Solid Tumors, version 1.1. (RECIST 1.1) (Figure 8). The patient was in good general condition with Eastern Cooperative Oncology Group (ECOG) performance status 1.

The patient was offered genetic testing which he accepted. Analysis of the *TP53* was performed by sequencing genomic DNA isolated from peripheral blood leukocytes. A pathogenic, heterozygous germline mutation *TP53* gene on exon 5, codon 151, CCC >ACC, c.451C >A (p.Pro151Thr) was identified. However, the patient did not give his consent for this test to be performed on his 8-year-old daughter.

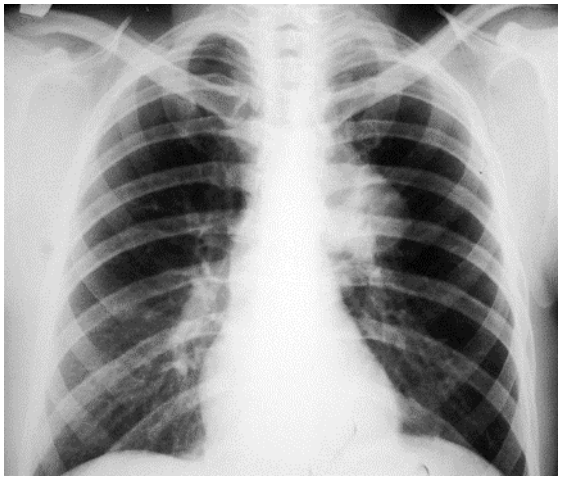

Fig. 6 – Chest radiography shows a tumor in the left lung.

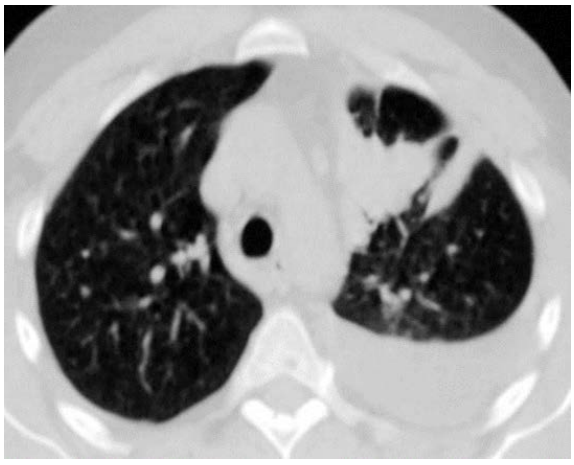

Fig. 7 – Chest computed tomography scan shows a tumor in the left lung with pleural effusion.

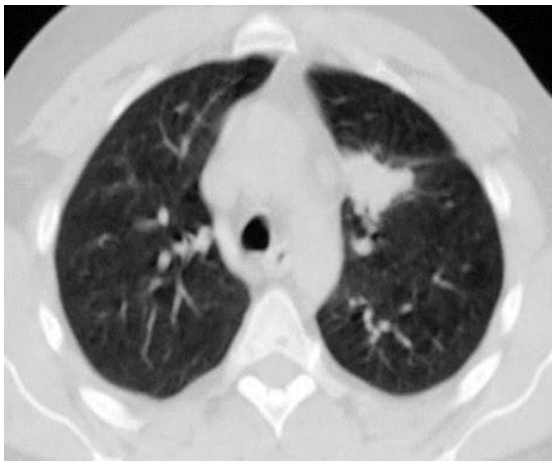

Fig. 8 – Chest computed tomography scan shows partial response to treatment.

Discussion

LFS is a rare autosomal-dominant inherited syndrome containing a germline mutation in the *TP53* gene, which predisposes to oncogenesis.

Furthermore, there is a hereditary condition of cancer predisposition that has been called LFS-like syndrome (LFL), which is defined as proband with any childhood cancer, adrenocortical tumor, brain tumor, or sarcoma in people under 45 years of age, the first or second-degree relative in the same lineage with LFS tumor at any age, and the first or second-degree relative in the same lineage with any type of malignancy before the age of 60 [8]. Approximately 70% of families affected by classical tumors carry germinal mutations in *TP53*. However, 40% of patients with LFL phenotype (families with other malignancies, different from classical tumors) carry *TP53* deleterious mutations. *TP53* mutations, associated with LFS or LFL, are mainly found in the DNA binding domain. Only a few cases of *TP53* mutations are out of this hotspot location [9, 10]. Looking into complete family history over multiple generations is very important as it could suggest an increased risk of cancer occurrence within a family. Likewise, it is equally important to regularly update the family history as the risk factor can change over the years. In our case, the mutation was found in the father who did not know about the *TP53* mutation until he was 49 years old. His variant of mutation is classified as likely pathogenic according to the ClinVar database [11]. The father was the only family member genotyped. His mother and two daughters probably had a mutation due to the early and rare cancers from which they died. The reported case describes the appearance of cancer at a young age, the presence of multiple malignancies in the same person, and the occurrence of rare malignancies within the same family. After the diagnosis of malignant disease, presented patients were classified and treated according to current oncology guidelines and recommendations [12–14]. Assessment of the effects of anticancer treatment was done according to Response Evaluation Criteria in Solid Tumors-RECIST 1.1 [15]. The performance status was assessed based on Eastern Cooperative Oncology Group-ECOG [16]. Despite the obvious connection between the reported patients, the diagnosis of LFS was made only after the confirmation of a malignant disease in the father. There were several reasons for this. First of all, LFS is rare, with an incidence of 1–9 : 100,000 inhabitants. The second reason was that all members of this family had malignancies of different primary sites and were treated in different medical institutions and countries. Moreover, the father probably did not pay enough attention to family history after two of his daughters were diagnosed with cancer. The third possible reason was the father's refusal of genetic testing for LFS even after his second daughter was diagnosed with two malignant diseases at an early.

Detection of *TP53* mutation in the third 8-year-old daughter could have led to inclusion in a screening program for early cancer detection which would significantly increase the possibility of longer survival. In our case, despite being made aware of this, the family did not agree to genetic testing for the third daughter. They believe that knowledge of the existence of LFS would create psychological pressure, which they are not ready to deal with. We hope the family will agree to genetic testing in the near future because early diagnosis leads to earlier and more effective treatment. This case also confirms both the need to provide patients with psychological support as well as information about the significance of regular diagnostic procedures in order to achieve the best possible outcome. These screening protocols recommend that carriers of the mutation should perform an abdominal ultrasound every 3–4 months, an annual MRI of the whole body, and an annual brain MRI (the first with gadolinium enhancement) from the first year of life. In addition, female carriers should perform an annual breast MRI from age 20. The possibility of a mastectomy that reduces the risk of cancer can be discussed, depending on the case [1]. However, a lot of current strategies using small molecule drugs to reactivate or modify dysfunctional *TP53* protein are being actively studied, but not yet in clinical trials with LFS patients [17].

Conclusion

Screening for the early manifestation of malignant disease is the key to prolonged survival in people with LFS. People with LFS require education about this genetic disorder, the types of malignancies they have an increased risk of, and the signs and symptoms of these diseases. Reproductive options for those in fertile age should be discussed. A periodic physical examination, recommended to be done annually, should be performed, including skin, breast, and neurological assessments. In the future, gene therapy will give optimism for the possibility of longer survival.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Frebourg T, Bajalica LS, Oliveira C, Magenheim R, Evans DG.* European Reference Network GENTURIS. Guidelines for the Li-Fraumeni and heritable TP53-related cancer syndromes. Eur J Hum Genet 2020; 28(10): 1379–86.
2. *de Andrade KC, Frone MN, Wegman-Ostrosky T, Khincha PP, Kim J, Amadou A,* et al. Variable population prevalence estimates of germline TP53 variants: a gnomAD-based analysis. Hum Mutat 2019; (40): 97–105.
3. *Li FP, Fraumeni JF.* Soft-tissue sarcomas, breast cancer, and other neoplasms – a familial syndrome? Ann Intern Med 1969; 71(4): 747–52.
4. *Malkin D, Garber JE, Strong LC, Friend SH.* CANCER. The cancer predisposition revolution. Science 2016; 352(6289): 1052–3.
5. *Druker H, Zelley K, McGee RB, Scollon SR, Kohlmann WK, Schneider KA,* et al. Genetic counselor recommendations for cancer

predisposition evaluation and surveillance in the pediatric oncology patient. Clin Cancer Res 2017; 23(13): e91–e97.
6. *Guha T, Malkin D.* Inherited TP53 mutations and the Li-Fraumeni syndrome. Cold Spring Harb Perspect Med 2017; 7(4): a026187.
7. *Kratz CP, Achatz MI, Brugieres L, Frebourg T, Garber JE, Greer MLC,* et al. Cancer screening recommendations for individuals with Li-Fraumeni syndrome. Clin Cancer Res 2017; 23(11): e38–e45.
8. *Bakhuizen JJ, Velthuizen ME, Stehouwer S, Bleiker EM, Ausems MG.* Genetic counselling of young women with breast cancer for Li-Fraumeni syndrome: a nationwide survey on the experiences and attitudes of genetics professionals. Fam Cancer 2019; 18(2): 231–9.
9. *Etzold A, Schröder JC, Bartsch O, Zechner U, Galetzka D.* Further evidence for pathogenicity of the TP53 tetramerization domain mutation p.Arg342Pro in Li-Fraumeni syndrome. Fam Cancer 2015; 14(1): 161–5.
10. *Macedo GS, Araujo Vieira I, Brandalize AP, Giacomazzi J, Inez Palmero E, Volc S.* Rare germline variant (rs78378222) in the TP53 3' UTR: evidence for a new mechanism of cancer predisposition in Li-Fraumeni syndrome. Cancer Genet 2016; 209(3): 97–106.
11. National Center for Biotechnology Information. ClinVar. Available from: https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000012369.
12. *Louis DN, Ohgaki H, Wiestler OD, Cavenee WK.* World Health Organization Histological Classification of Tumours of the Central Nervous System. Lyon, France: International Agency for Research on Cancer; 2016.
13. *Detterbeck FC, Boffa DJ, Kim AV, Tanoue LT.* The Eighth Edition Lung Cancer Stage Classification. Chest 2017; 151(1): 193–203.
14. *Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman JR, Bharat A,* et al. Non-Small Cell Lung Cancer, Version 3.2022, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2022; 20(5): 497–530.
15. *van Persijn van Meerten EL, Gelderblom H, Bloem JL.* RECIST revised: implications for the radiologist. A review article on the modified RECIST guideline. Eur Radiol 2010; 20(6): 1456–67.
16. *Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET,* et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982; 5(6): 649–55.
17. U.S. National Library of Medicine. ClinicalTrials.gov 2021. Treatment of Carrying TP53 Harmful Mutations in Advanced Refractory Solid Tumors. Identifier NCT03645200.

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CASE REPORT

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Juvenile gigantomastia: subcutaneous mastectomy with primary reconstruction

Juvenilna gigantomastija: supkutana mastektomija sa primarnom rekonstrukcijom

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Abstract

Introduction. Gigantomastia is one of the most severe anomalies of the female breasts and is caused by their pathological enlargement. Juvenile gigantomastia (JG) of the breasts is a rare disease of an unknown etiology characterized by a sudden and alarmingly rapid, continued growth of the breasts in puberty. **Case report.** We present two patients with massive bilateral JG. Both patients had normal hormonal status and denied any other health issues, including a positive family history of gigantomastia. The skin overlying the breasts was red, without ulcerations, and with visibly enlarged superficial veins. The nipples were not well defined from the surrounding skin and were positioned below the level of the umbilicus. Patients were successfully treated with a surgical technique consisting of a bilateral subcutaneous mastectomy with the primary reconstruction of the breasts using polyurethane implants and reconstruction of the nipple by the free nipple graft technique. The histopathological reports from both patients' biopsy specimens presented diffuse hyperplasia of the glandular and stromal tissue. Both patients had excellent esthetic results with minimally visible postoperative scars. **Conclusion.** Surgical management of JG is the primary means of treatment. This paper presents significant results and effects of plastic surgery, and the applied surgical method can be recommended for the successful management of JG.

Key words:
gigantomastia; plastic surgery procedures; treatment outcome.

Apstrakt

Uvod. Gigantomastija predstavlja jednu od najozbiljnijih anomalija ženskih dojki, a nastaje usled njihovog patološkog uvećanja. Juvenilna gigantomastija (JG) dojki je retka bolest, nepoznate etiologije, koju karakteriše nagli i alarmantno brzi, kontinuirani rast dojki u pubertetskom periodu. **Prikaz bolesnika.** Prikazujemo dve bolesnice sa masivnom bilateralnom JG. Obe bolesnice imale su normalan hormonski status i negirale bilo kakve druge zdravstvene probleme, uključujući porodičnu istoriju gigantomastije. Koža grudi obe bolesnice bila je crvena, bez ulceracija, sa vidno uvećanim površnim venama. Bradavice nisu bile jasno definisane u odnosu na okolnu kožu i bile su postavljene ispod nivoa pupka. Bolesnice su uspešno lečene hirurškom tehnikom koja je uključivala bilateralnu supkutanu mastektomiju sa primarnom rekonstrukcijom dojki poliuretanskim implantatima kao i rekonstrukciju bradavice tehnikom slobodnog grafa. U histopatološkim nalazima iz uzoraka biopsije obe bolesnice nađena je difuzna hiperplazija žlezdanog i stromalnog tkiva. Obe bolesnice imale su odlične estetske rezultate sa minimalno vidiljvim postoperativnim ožiljcima. **Zaključak.** Hirurško lečenje je primarno sredstvo u lečenju JG. Prikazani su značajni rezultati i efekti plastične hirurgije, a primenjeni hirurški metod može se preporučiti za uspešno lečenje JG.

Ključne reči:
hirurgija, plastična, procedure; gigantomastija; lečenje, ishod.

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Introduction

Deformities of the breasts have a cumulative psychologically negative effect on the female population that causes a feeling of decreased femininity and sexual unattractiveness. They try to hide the defects through different ways of dressing and seclusion, which in turn has an effect on their normal activities of everyday living.

The possibility of reconstruction gives hope to the patient undergoing mastectomy in terms of decreasing the possible physical and emotional effects of undergoing the procedure. The shape and symmetry of the breasts are deemed more significant than the visibility of the postoperative scars. Having a plan for the treatment of gigantomastia is crucial for the patients facing the diagnosis. It is essential for surgeons to understand the history of breast reconstruction, as it is the core of the comprehensive and all-inclusive approach to healing.

Abnormalities of female breasts have been a rising concern for many centuries. That has given a rise to what is known and performed as a reduction mammoplasty, viewed both as a science and an art form. This reduction in breast volume is performed to either alleviate clinical symptoms or improve positive body image in patients.

In the seventh century, a Greek physician, Paulus Aegina, first described breast reduction for treating gynecomastia. In 1561, a German physician Hans Schaller reported using breast amputation for treating gigantomastia. Later, in 1669, a British physician Briton William Durston reported using breast reduction for breast hypertrophy [1].

Diseases of the breasts differ extremely in their clinical presentations and mechanisms of onset and always cause a certain amount of doubt in clinicians who deal with breast pathology.

The literature describes five subtypes of gigantomastia: juvenile (also known as pubertal or virginal), gestational, idiopathic, penicillin-related, and obesity-related.

Diffuse hypertrophy of the breasts in the adolescent period, which presents either before or after the onset of menarche, is known as juvenile or virginal hypertrophy and, in extreme cases, as JG. It is defined as the extreme enlargement of the breasts, most commonly within a 6-month period, which is further followed by a continual period of gradual breast enlargement [2].

JG is a rare disorder (0.4%) characterized by excessive breast tissue enlargement and proliferation, accompanied by serious physical and psychosocial consequences. It is sporadic in nature; however, in written works, it is also described as having a positive family background [3–6].

The definition of JG is not universally accepted in literary works. It is most commonly described as an increase in breast size, which occupies over 3% of the full body mass or excess breast tissue weighing over 1.5 kg [7].

Kulkarni et al. [8] defined JG as an etiologically undefined benign progressive bilateral breast enlargement to the extent that the only means of treatment is with a surgical breast reduction, during which more than 1,800 g of breast tissue would be removed from each side, respectively.

The first case of gigantomastia described in medical literature was in 1670 in Plymouth [9]. Kupfer et al. [4] stated that the first published case of hypertrophy was in 1919 by Henry Albert.

JG can be either unilateral or bilateral in presentation, as well as either symmetric or asymmetric.

The leading characteristic of JG is that at some undetermined point during adolescence, the breasts begin to enlarge extremely and rapidly, followed by stretching of the overlying skin and a dark red discoloration; recurring mastitis and nipple deformation are also frequently present. In some cases, sudden enlargement of the breasts can also lead to ulceration of the overlying skin of the breasts.

The etiology of the onset of JG is still unclear. The main hypothesis is that the cause is a secondary hypersensitivity to certain hormones in the adolescent period, such as estrogen (ER), PR, prolactin, or growth factors. However, most of the reported cases in medical journals and other literature stated that normal hormonal levels were present [8–14]. It is also noted in the literature that some autoimmune diseases, such as systematic erythematous lupus, are linked to gigantomastia [15, 16].

In the following cases, the patients were presented to a council of specialists which consisted of a plastic surgeon, general surgeon, radiologist, endocrinologist, pathologist, pediatrician, and psychologist. Due to the nature of their clinical presentations, with large masses of fibrocystic breast tissue, as well as the excess distance between the nipples, it was decided that a subcutaneous mastectomy would be performed under general anesthesia. That would be executed with skin-sparing mastectomy, as done in the inverted-T surgical technique with reduction mammoplasty, followed by a primary reconstruction of the breasts with polyurethane breast implants and reconstruction of the areola with a free nipple transplant in the same surgical act.

It is important to state that both patients gave their full, conscious, and informed consent to be included in this case report.

Case 1

A 19-year-old Caucasian nulliparous female patient presented with slightly asymmetric bilateral JG. The patient stated that the additional growth of her already-developed breasts was noticed a year ago. In the beginning, the enlargement was both symmetric and moderate. Six months ago marked the onset of extreme breast growth, with a subtly more pronounced enlargement of the left breast.

The patient stated that the onset of menarche was at the age of 12 and that it has been since accompanied by regular menstrual cycles lasting 28 days. She denied chronic illnesses, medication use, and allergies to food and medications. The patient underwent spinal surgery for scoliosis at the age of 13.5. She negated positive family history of gigantomastia or macromastia.

The patient stated that before the onset of the described breast growth, her breasts fit a C-cup in bra size and that, in just a year, they enlarged to the presenting size. The enlarged

breasts posed as both a physical burden, causing back pain, frequent mastitis, and restriction in everyday activities, as well as a psychological burden including but not limited to social isolation and withdrawal from peer groups. Due to significant bilateral mastitis a month before the surgery, the patient was on a two-week triple antibiotic therapy.

Upon clinical examination, it was noted that there was an asymmetric hypertrophy of both breasts, with a slightly larger left breast. With the patient in the upright standing position, the breasts reached the level below the umbilicus. The skin overlying both breasts was intact, without ulcerations, distended, and dark red with a perceivably evident and dilated venous presentation. In both breasts, it was recognized that the nipples were deformed, atrophied, and inflamed (Figures 1A and 1B). On bimanual palpation, the breasts were hard and nodular. The distance between the midline and the nipple was 58 cm on the right breast and 63 cm on the left.

Hormonal levels of ER, PR, testosterone, prolactin, luteinizing hormone (LH), and follicle-stimulating hormone (FSH), as well as tumor marker analysis for CA-125, all came back within the normal physiological ranges. The patient was tested for systemic lupus, diabetes, and diseases of the thyroid gland. All test results were negative.

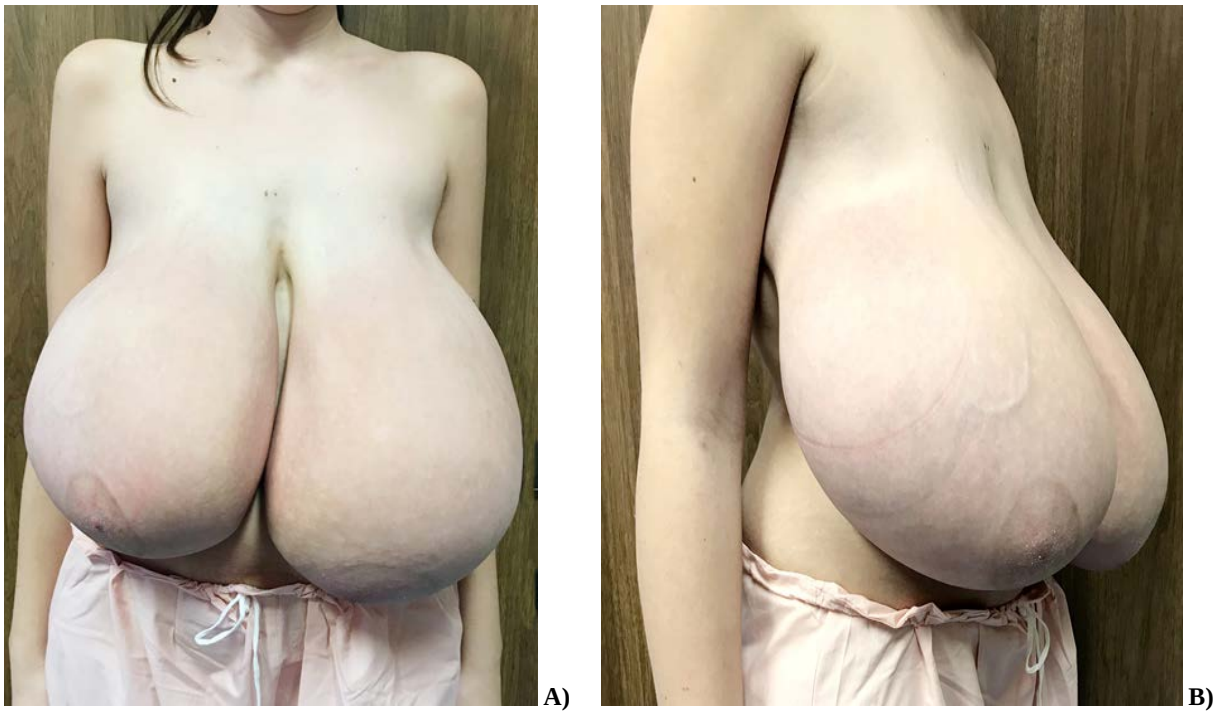
Ultrasound (US) of the breasts described that both of the breasts were extreme in volume, with a glandular parenchymal structure. Bilaterally, in the parenchyma, there were multiple large hypoechogenic lesions with a detectable Cd signal. The largest one is located in the left breast at the fusion of the upper quadrants towards the left upper quadrant (LUQ), spanning a longitudinal diameter of 10 cm. US-guided core biopsy was performed, and one sample was obtained and sent for pathology analysis. In the right breast in the upper right quadrant (URQ), the largest focal lesions spanned a diameter of 6 cm. Two core biopsy samples were obtained and sent for pathology analysis and report. The breast parenchyma was diffusely oedematous. There were no pathologically altered lymph nodes (LN) in the axillae. The pathology report stated that the samples were made up of focally multiplied connective tissue, which incorporated ducts and lobules of the breasts, coated in a uniform epithelium. Multiple microcalcifications were noted. There was no tumorous tissue in the samples.

Based on the medical exam and a detailed radiological and endocrinologic exam of the patient, a uniform decision was reached by the council of specialists for a bilateral subcutaneous mastectomy with a primary reconstruction with implants. The patient gave full and informed consent for the surgical intervention.

The tissue removed from the right breast weighed 6,300 g, and the left 6,800 g (Figures 2A and 2B).

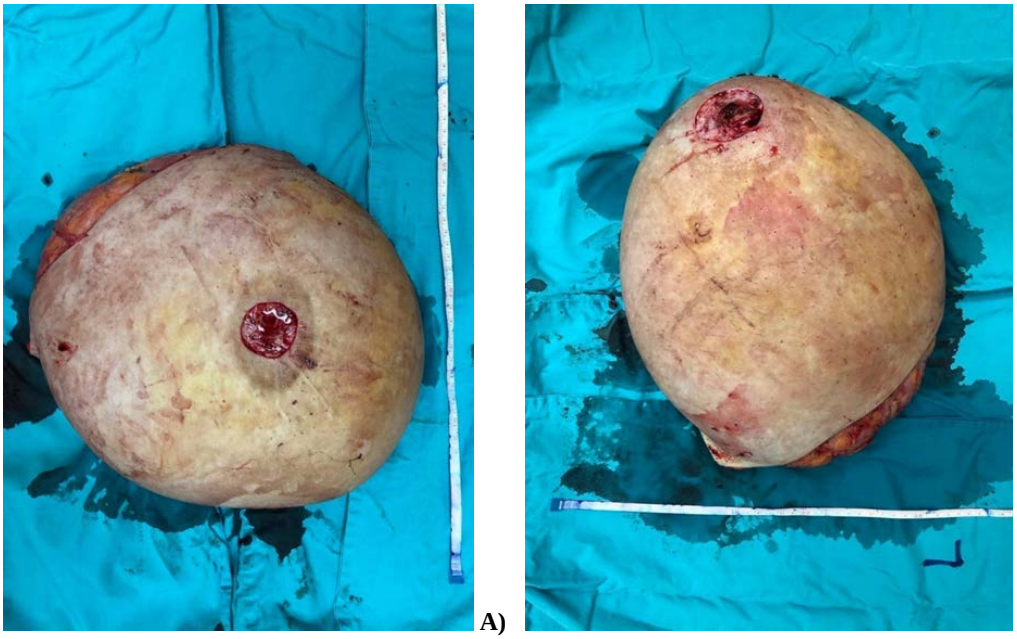
In the immediate postoperative period, the patient was not anemic, and no other frequently associated postoperative complications presented.

The pathology report defined the benign tissue sample as a secondary extensive proliferation of the glandular and stromal tissue. There was no chronic inflammatory cell infiltration nor histopathological confirmation of malignancy. Testing for ER and PR receptors was negative, which allowed for the omission of using tamoxifen in the postoperative period.



A) **B)**

Fig. 1 A) and B) – Slightly asymmetric hypertrophy of both breasts in a 19-year-old patient with a larger left breast. In both breasts, the nipples are deformed, atrophied, and inflamed. The skin overlying both breasts is distended and dark red with a visible dilated venous presentation.



A) **B)**

Fig. 2 – Tissue removed from the breasts of the first presented patient: A) from the right breast, weighing 6,300 g and B) from the left, weighing 6,800 g.

Case 2

A 16-year-old Caucasian nulliparous female patient presented with extreme asymmetric bilateral JG. The patient stated that further growth of already-developed breasts was noticed. In the beginning, the enlargement was asymmetric and moderate; however, ten months ago marked the onset of extreme breast growth, with considerably pronounced growth of the left breast.

The patient stated that the onset of menarche was at the age of 11 and that it was since followed by regular menstrual cycles lasting 28 days. The patient denied chronic illnesses, medication use, and allergies to food and medications. She negated positive family history of gigantomastia or macromastia; however, she stated that her mother had a fibroadenoma of the breast.

When we encountered the patient, she noted and presented previous medical documentation stating that she had previously had two partial resections of the left breast with seven months in between. Following each of the resections, the enlargement continued at a rapid pace.

The patient stated that before the onset of the described breast growth, her breasts were a C-cup in bra size and that in the course of 22 months, the breasts enlarged to the presenting size. At the start of the clinical course of enlargement, the left breast began to enlarge and was erythematous in presentation. The patient was put on triple antibiotic therapy for mastitis. This problem recurred multiple times, and each time, the patient was put on the same antibiotic therapy, which alleviated the symptoms short term. That was followed by a subsequent enlargement of the right breast. The enlarged breasts posed as both a physical burden causing back pain, frequent mastitis, and restriction in everyday activities, and a psychological burden including but not limited to social isolation and withdrawal from peer groups.

Upon examination, it was noted that there was an extreme asymmetric hypertrophy of both breasts, with a significantly larger left breast, which in the upright standing position reached below the umbilicus. The skin overlying both breasts was intact, without ulcerations, strained, dark red with a visually evident and dilated venous presentation. In both breasts, it was noted that the nipples were deformed, atrophied, and inflamed (Figures 3A and 3B). On palpation, the breasts were hard and nodular. The distance between the midline and the nipple was 37 cm on the right breast and 58 cm on the left.

Hormonal levels of ER, PR, testosterone, prolactin, LH, FSH, as well as tumor marker analysis for CA-125, all came back within the normal physiological ranges. The patient was tested for systemic lupus, diabetes mellitus, and diseases of the thyroid gland. All test results were negative.

US of the breasts stated that the breasts were voluminous, with the left breast being substantially more voluminous than the right. The breasts were of a glandular composition, hypoechogenic. The left breast showed hypertrophy with numerous hypoechogenic zones clearly demarcated from the surrounding tissue between the connective tissue septa, which could differentially be diagnosed most likely with gigantocellular fibroadenoma, the largest zone spanning up to 2.5 cm. In the right breast in the LUQ, a hypoechogenic solid lesion spanning 2 cm and, next to it, a smaller one spanning 0.8 cm were present. At the fusion with the right quadrant, two hypoechogenic solid lesions spanning 2.1 cm and 1.8 cm, respectively, were noted. Ductal (canals) had a width of up to 3 mm, without clear differentiation of intraluminal proliferation. There were no enlarged or pathologically differentiated LN in the axillae.

Magnetic resonance imaging (MRI) of the breasts was performed with and without contrast. It stated that the structure of the breasts was fibroglandular. Bilaterally there were no suspicious tumorous lesions, nor was there a pathological post-contrast increase in signal intensity. The left breast showed a significantly increased cutaneous thickness. No morphological or dynamic MRI signs of malignancy were noted. Biopsy samples taken from the breasts came back with a diagnosis of multiple giant fibroadenomas.

Based on the medical exam, pediatrician consultation, and a detailed radiological and endocrinologic exam of the patient, a uniform decision was reached by the council of specialists for a bilateral subcutaneous mastectomy with a primary reconstruction with implants. Due to the patient's age, her parents gave full and informed consent for the surgical intervention.

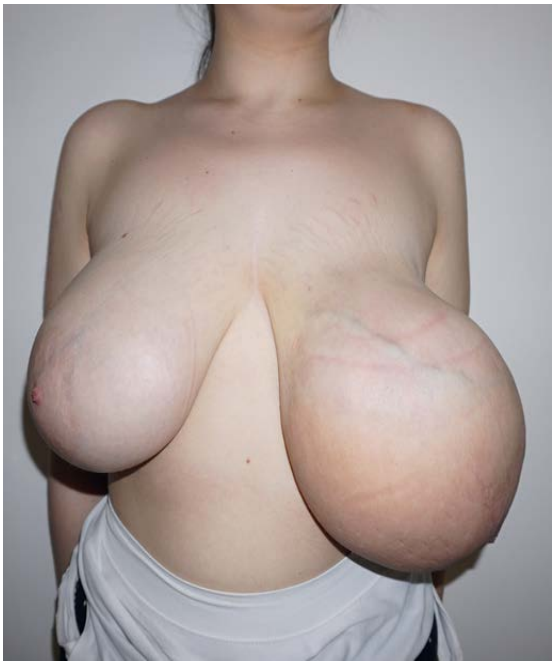
The tissue removed from the right breast weighed 2,600 g, and from the left, 4,100 g (Figures 4A and 4B).

In the immediate postoperative period, the patient was not anemic, and no other frequently noted postoperative complications presented (Figures 5A and 5B).

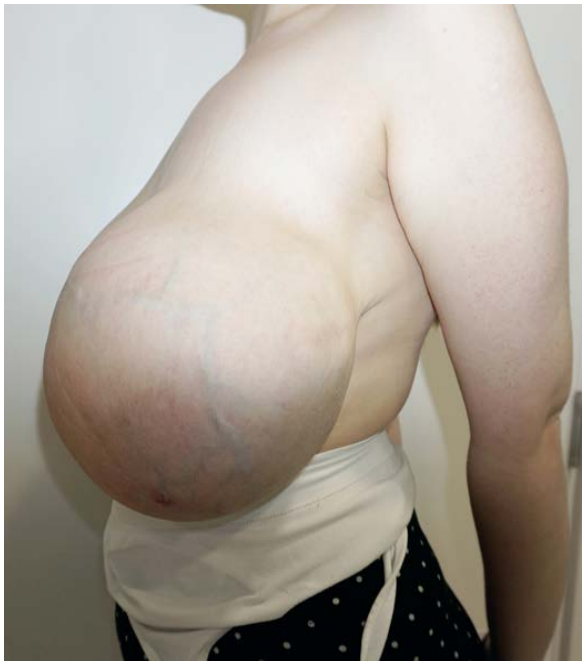
The pathology report defined the benign tissue sample as the secondary extensive proliferation of glandular and stromal tissue. There was no chronic inflammatory cell infiltration nor histopathological confirmation of malignancy. Testing for ER and PR receptors was negative, which allowed for the omission of using tamoxifen in the postoperative period.

Our surgical technique included a subcutaneous mastectomy with nipple reconstruction as free transplants (Figures 6A and 6B).

The patients were satisfied with the esthetic outcome of their individual surgery (Figures 7 and 8).

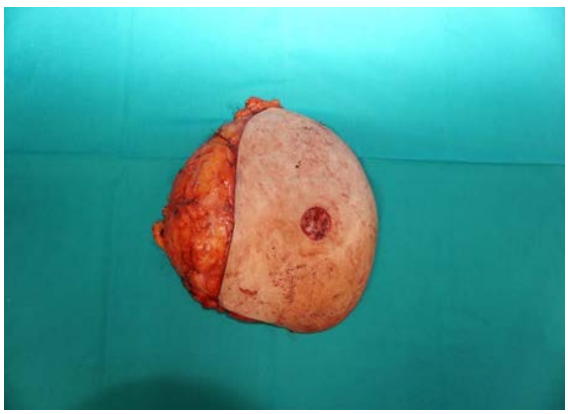


A)

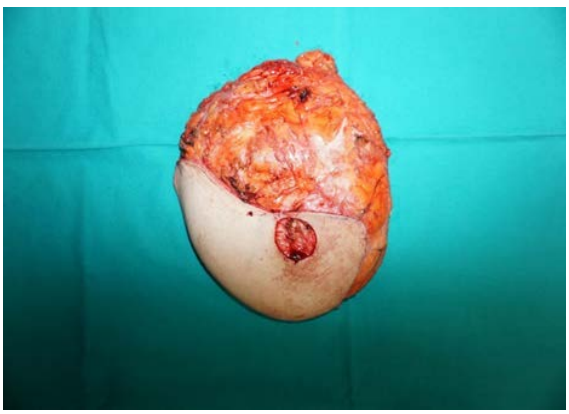


B)

Fig. 3 A) and B) – Extremely asymmetric hypertrophy of both breasts in a 16 year-old-patient. Skin overlying both breasts is dark red with evident and dilated venous presentation. Nipples are deformed, atrophied, and inflamed.

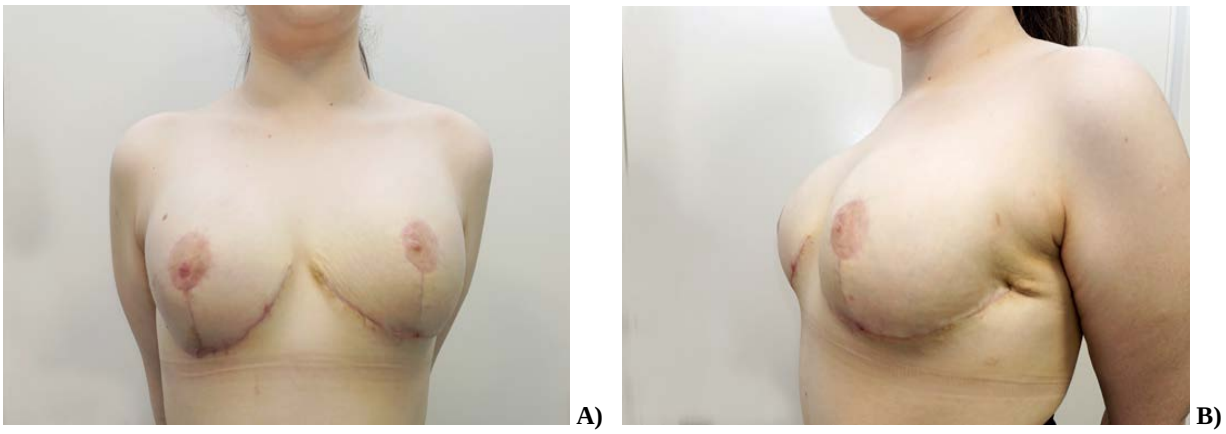


A)



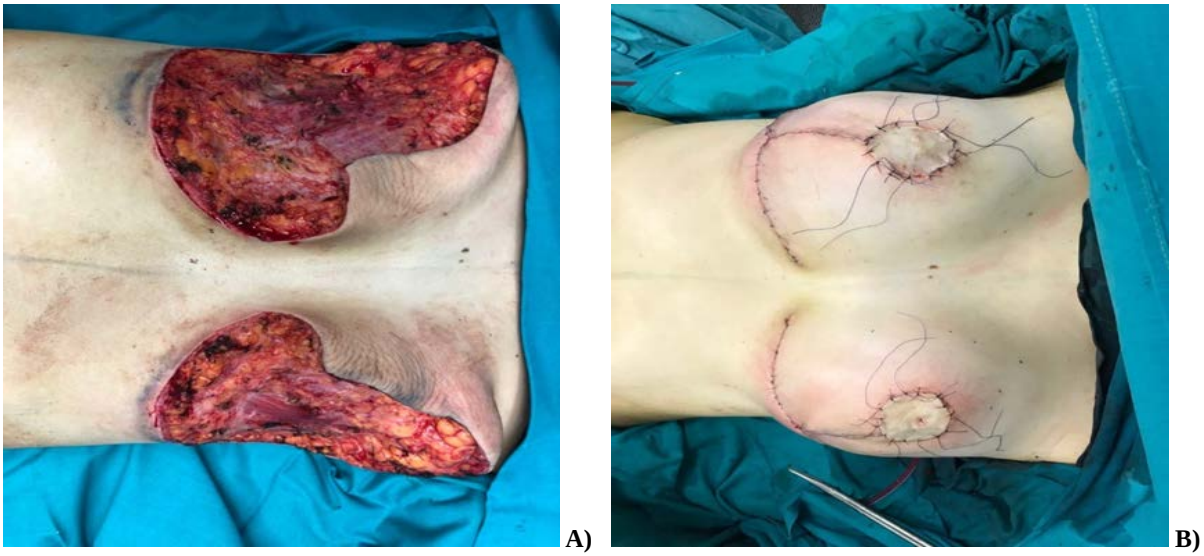
B)

Fig. 4 – Tissue removed during mastectomy from the breasts of the second presented patient: A) from the right breast, weighing 2,600 g and B) from the left, weighing 4,100 g.



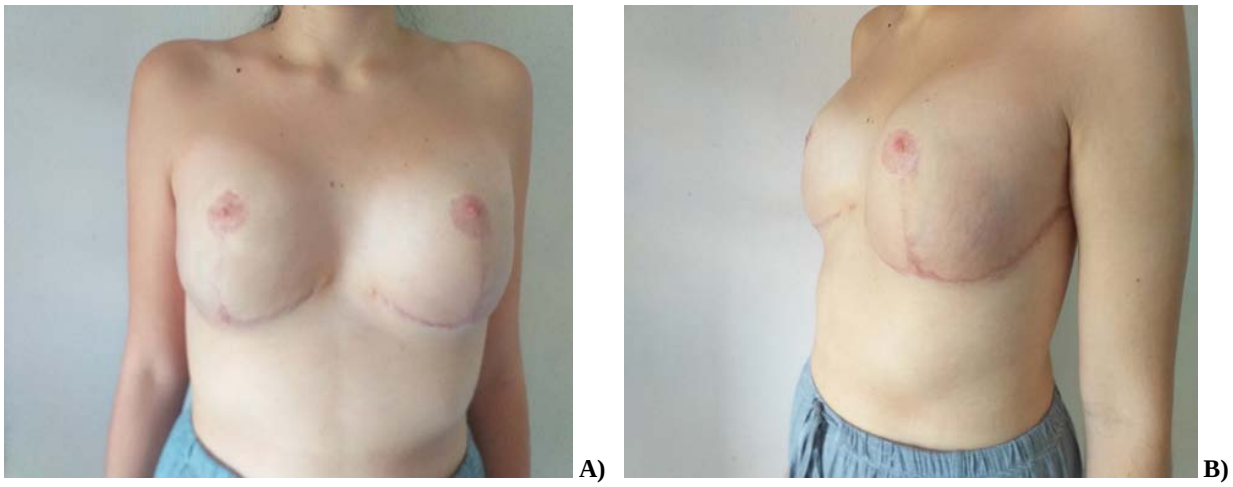
A) B)

Fig. 5 A) and B) – Appearance of the patient in the immediate postoperative period: she was not anemic and no complications presented.



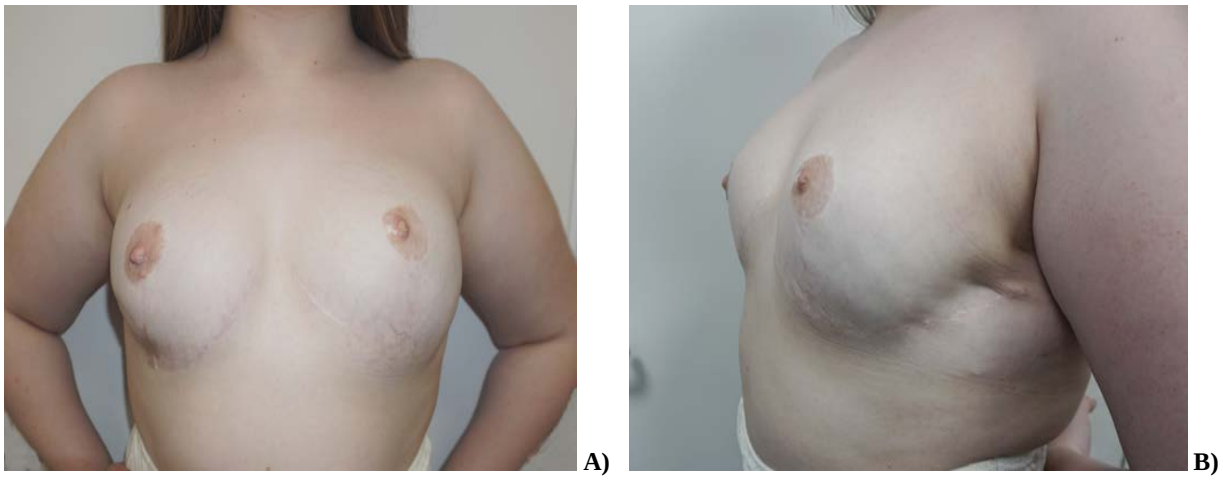
A) B)

Fig. 6 A) and B) – Appearance of the patient on whom the surgical technique was performed including a subcutaneous mastectomy with nipple reconstruction as free transplants.



A) B)

Fig. 7 A) and B) – Aesthetic outcome in the first operated patient.



A) B)

Fig. 8 A) and B) – Aesthetic outcome in the second operated patient.

Discussion

The exact etiology of JG is not fully understood. Contemporary theories describe the sensitivity of the receptors in the breasts to normal levels of circulating ER, increased local or generalized levels of ER, or expression of the PR receptors, including some congenital and autoimmune diseases.

Recently, genetic background to phosphatase and tensin homolog tumor-suppressor (*PTEN*) gene has been assigned. In 2002, Li et al. [16] demonstrated using live mice experiments that mutation and deletion of the *PTEN* gene are connected to increased lobuloalveolar and ductal growth, delayed involution, and hyperproliferation of the epithelium of the breast glandular tissue; however, the clinical analyses have not confirmed this [17].

Our patients had specific and prominent clinical presentations of JG. They did not have positive family histories or any correlation with chronic and autoimmune diseases. Analysis of the *PTEN* gene mutation was not performed as there is still a significant lack of validated and current guidelines and recommendations for *PTEN* testing [18]. Both presented patients had normal endocrinological status both pre- and postoperatively.

The clinical characteristics of JG in the presented patients were similar compared to other types of gigantomastia. Their physical, psychological, and social consequences included bodily symptoms of back and neck pain and avoiding socialization due to extreme breast size. The patients also presented with chronic intertrigo resistant to topical therapy underneath the breasts, grooving on the shoulders from excess weight supported by the brassiere, and numbness and tingling sensation in the arms and fingers. The patient's body mass index (BMI) was in the overweight range due to the enormous weight occupied by the enlarged breasts.

We applied the Mosteller formula: body surface area (m^2) = [height (cm) × weight (kg)] / 3,600)½ [19], which calculates the patient's body surface area and takes into account their presenting height and weight preoperatively. The Schnur Sliding Scale (SSS) uses the Mosteller formula to break down the body surface area and give us the exact amount of minimal breast tissue to remove. The patient from the first case was 180 cm tall and weighed 86 kg with a BMI of 26.5. The Mosteller formula for her calculated that her body surface area (BSA) was 2.11 m^2. SSS defined the minimal amount of breast tissue to be removed at 750 g per breast. The patient from the second case was 170 cm tall and weighed 74 kg with a BMI of 25.6. The Mosteller formula for her calculated that her BSA was 1.87 m^2. SSS defined the minimal amount of breast tissue to be removed at 482 g per breast.

Prior to consulting plastic surgery specialists, the patients had undergone routine diagnostic procedures, including biopsies, under the care and referral of oncologists and presented the stated medical documentation. Histological analysis of the specimen is the only definitive means of establishing a proper diagnosis, where the normal glandular-alveolar development of the normal breast tissue is significantly surpassed by ductal proliferation and stromal alteration that presents with this diagnosis.

In the differential diagnosis of JG, we have to exclude benign changes such as giant fibroadenomas and phyllodes tumors, malignant tumors such as lymphomas and sarcomas, pseudo-gigantomastia associated with obesity, and breast hypertrophy due to various endocrine disorders [20].

JG can be treated in four ways: surgical management, pharmacological therapy given preoperatively or postoperatively, and sole pharmaceutical management.

In the case of surgical management, the choices are subcutaneous mastectomy and primary reconstruction with implants, and various techniques of reduction mammoplasty. The nipples can be reconstructed on a vascular pedicle or as free transplants.

Our surgical technique included a subcutaneous mastectomy with nipple reconstruction as free transplants, which was based on research by Hoppe et al. [21], who claimed a significant correlation ($p < 0.01$) and coefficient quota 7.0 for the probability of a recurrence using reduction mammoplasty compared to mastectomy [21] and the research by Fiumara et al. [22], who published statistical proof that reconstruction of the nipples as free transplants leads to a reduction in the possibility of recurrence compared to those reconstructed on a vascular pedicle ($p = 0.005$). We adapted our surgical tech-

nique and avoided inferior de-epithelialization of the flap and serratus muscle flap due to the characteristics of polyurethane implants that we used.

Tamoxifen is a selective ER modulator used as the first line in medicament treatment as a conservative treatment in juvenile hypertrophy of the breasts [23]. Conservative treatment shows the possibility of stopping progression and causing slight regression of the disease; however, it is incapable of restoring the breasts to their original size if not used in conjunction with other treatment options. It is stated that tamoxifen is given as a means to decrease the recurrence of disease in breast reduction [24]. There is little evidence of the efficacy of tamoxifen; its long-term effects, as well as its safety, are unknown. The known side effects are an increased risk of endometrial cancer, thromboembolism, hot flashes, and a decrease in bone density [2, 21, 25]. Due to the complete removal of all glandular tissue in the breasts, negative ER and PR tissue receptors, there was no need to treat our patients with tamoxifen postoperatively.

Both patients wore compressive bras for a month following the surgery, and they were advised to keep wearing a bra daily as part of their everyday attire. Two weeks following the surgery, the patients presented their breasts with healed nipples, minimal scarring, and no wound infection or ulceration. At the same appointment, the sutures were removed, and the patients were told to hydrate their scars and to start applying topical scar treatment ointment to improve the appearance of their operative scars. The patients were asked to refrain from physical activity for the first postoperative month and to sleep on their backs. At the one-month mark, the patients were showcased how to massage their breasts to help with the adaption of the tissue and its placement over the implant. Three months following the surgery, the entire reconstruction was very satisfactory in appearance. Surgical management had an enormously positive influence on the physical and psychological status of the patients, and with time they could return to their everyday activities.

Complications following mastectomy have been described in the literature as early and late surgical complications, as well as local and systemic complications. They comprise bleeding, swelling, hematomas, seromas, disruption of the wound, dermatitis, nipple graft complications, lymphedema, scar and nipple discoloration, wound infection, etc. [26].

We followed the patients for the first two years with the following appointment schedule – two weeks postoperatively, one month, three months, six months, one year, and two years, respectively. Postoperative US was done at six-month intervals, which evaluated the implant placement and the continuity of the physical state of the implant.

In both cases, the patients were satisfied with the esthetic outcome, of their individual surgery.

Conclusion

Bilateral subcutaneous mastectomy with the primary reconstruction of the breasts using polyurethane implants and reconstruction of the nipple by the free nipple graft technique can be recommended for the successful management of JG with a very satisfying esthetic result.

REFERENCES

1. *Dieffenbach JF.* Die extirpation der bruestdruese. In: *Dieffenbach JF*, editor. Die Operative Chirurgie. Leipzig: Brockhaus; 1848. p. 359–73. (German)
2. *Wolfswinkel EM, Lemaine V, Weathers WM, Chike-Obi CJ, Xue AS, Heller L.* Hyperplastic breast anomalies in the female adolescent breast. Semin Plast Surg 2013; 27(1): 49–55.
3. *Zargar AH, Laway BA, Masoodi SR, Chowdri NA, Wani AI.* Unilateral gestational macromastia-an unusual presentation of a rare disorder. Postgrad Med J 1999; 75(880): 101–3.
4. *Kupfer D, Dingman D, Broadbent R.* Juvenile breast hypertophy: Report of a familial pattern and review of the literature. Plast Reconstr Surg 1992; 90(2): 303–9.
5. *Govrin-Yehudain J, Kogan L, Cohen HI, Falik-Zaccai TC.* Familial juvenile hypertrophy of the breast. J Adolesc Health 2004; 35(2): 151–5.
6. *Netscher DT, Mosharrafa AM, Laucirica R.* Massive asymmetric virginal breast hypertrophy. South Med J 1996; 89(4): 434–7.
7. *Dafydd H, Roehl KR, Phillips LG, Dancey A, Peart F, Shokrollahi K.* Redefining gigantomastia. J Plast Reconstr Aesthet Surg 2011; 64(2): 160–3.
8. *Kulkarni D, Beechey-Newman N, Hamed H, Fentiman IS.* Gigantomastia: A problem of local recurrence. Breast 2006; 15(1): 100–2.
9. *Durston W.* An extract of a letter written to the publisher from Plymouth Novem. 2. 1669. by William Durston Dr. of Physick; concerning the death of the big-breasted woman (discoursed of in numb. 52.) together with what was thereupon observed in her body. Royal Soc (London) 1669; 4(45–56): 1068–9.
10. *Zhong A, Wang G, Yang J, Xu Q, Yuan Q, Yang Y,* et al. Stromal-epithelial cell interactions and alteration of branching morphogenesis in macromastic mammary glands. J Cell Mol Med 2014; 18(7): 1257–66.
11. *Pülzl P, Schoeller T, Tzankov A, Wechselberger G.* Unilateral breast enlargement 5 years after reduction mammaplasty. Aesthetic Plast Surg 2005; 29(5): 404–6.
12. *Wechselberger G, Pülzl P, Pichler M, Schoeller T, Piza-Katzer H.* Juvenile gigantomastia treated by reduction mammoplasty. Am J Surg 2004; 188(3): 333–4.
13. *Arscott GD, Craig HR, Gabay L.* Failure of bromocriptine therapy to control juvenile mammary hypertrophy. Br J Plast Surg 2001; 54(8): 720–3.
14. *Touraine P, Youssef N, Alyanakian MA, Lechat X, Balleyguier C, Duflos C,* et al. Breast inflammatory gigantomastia in a context of immune-mediated diseases. J Clin Endocrinol Metab 2005; 90(9): 5287–94.
15. *Duffy DA, Demers ML, Molin MR.* Systemic lupus erythematosus with breast gigantism. J Rheumatol 1995; 22: 1214–5.
16. *Li G, Robinson GW, Lesche R, Martinez-Diaz H, Jiang Z, Rozengurt N,* et al. Conditional loss of PTEN leads to precocious development and neoplasia in the mammary gland. Development 2002; 129(17): 4159–70.
17. *Koves IH, Zacharin M.* Virginal breast hypertrophy of an 11-year-old girl. Journal of paediatrics and child health 2007; 43(4): 315–7.
18. *Fusco N, Sajjadi E, Venetis K, Gaudioso G, Lopez G, Corti C,* et al. PTEN Alterations and Their Role in Cancer Management: Are

We Making Headway on Precision Medicine? Genes (Basel) 2020; 11(7): 719.
19. *Mosteller RD*. Simplified calculation of body-surface area. N Engl J Med 1987; 317(17): 1098.
20. *Güneş D, Mutafoğlu-Uysal K, Canda T, Saydam S, Cemeroğlu AP, Olgun N*. Unilateral juvenile (virginal) hypertrophy of the breast. Turk J Pediatr 2008; 50(3): 278–81.
21. *Hoppe IC, Patel PP, Singer-Granick CJ, Granick MS*. Virginal mammary hypertrophy: a meta-analysis and treatment algorithm. Plast Reconstr Surg 2011; 127(6): 2224–31.
22. *Fiumara L, Gault DT, Nel MR, Lucas DN, Courtauld E*. Massive bilateral breast reduction in an 11-year-old girl: 24% ablation of body weight. J Plast Reconstr Aesthet Surg 2009; 62(8): e263–6.
23. *Hisham A, Abd Latib M, Basiron N*. Juvenile Breast Hypertrophy: A Successful Breast Reduction of 14.9% Body Weight without Recurrence in a 5-Year Follow-Up. Case Rep Surg 2017; 2017: 3491012.
24. *Qin F, Si L, Zhang H, Zhang M, Zeng A, Long F,* et al. Management of gestational gigantomastia with breast reconstruction after mastectomy: case report and literature review. J Int Med Res 2020; 48(6): 300060520920463.
25. *Pruthi S, Jones KN*. Nonsurgical management of fibroadenoma and virginal breast hypertrophy. Semin Plast Surg 2013; 27(1): 62–6.
26. *Barton MB, West CN, Liu IL, Harris EL, Rolnick SJ, Elmore JG*, et al. Complications following bilateral prophylactic mastectomy. J Natl Cancer Inst Monogr 2005; (35): 61–6.

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

Blinder MA. Anemia and Transfusion Therapy. In: Ahya NS, Flood K, Paranjothi S, editors. The Washington Manual of Medical Therapeutics, 30th edition. Boston: Lippincot, Williams and Wilkins; 2001. p. 413-28.

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

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

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Literatura

U radu literatura se citira kao superskript, a popisuje rednim brojevima pod kojima se citat pojavljuje u tekstu. Navode se svi autori, ali ako broj prelazi šest, navodi se prvih šest i *et al*. Svi podaci o citiranoj literaturi moraju biti tačni. Literatura se u celini citira na engleskom jeziku, a iza naslova se navodi jezik članka u zagradi. Ne prihvata se citiranje apstrakata, sekundarnih publikacija, usmenih saopštenja, neobjavljenih radova, službenih i poverljivih dokumenata. Radovi koji su prihvaćeni za štampu, ali još nisu objavljeni, navode se uz dodatak „u štampi“. Rukopisi koji su predati, ali još nisu prihvaćeni za štampu, u tekstu se citiraju kao „neobjavljeni podaci“ (u zagradi). Podaci sa interneta citiraju se uz navođenje datuma pristupa tim podacima.

Primeri referenci:

Đurović BM. Endothelial trauma in the surgery of cataract. Vojnosanit Pregl 2004; 61(5): 491–7. (Serbian)

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

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

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

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

Tabele

Sve tabele pripremaju se sa proredom 1,5 na posebnom listu. Obeležavaju se arapskim brojevima, redosledom pojavljivanja, u levom uglu (**Tabela 1**), a svakoj se daje kratak naslov. Objašnjenja se daju u fus-noti, ne u zaglavlju. Svaka tabela mora da se pomene u tekstu. Ako se koriste tuđi podaci, obavezno ih navesti kao i svaki drugi podatak iz literature.

Ilustracije

Slikama se zovu svi oblici grafičkih priloga i predaju se kao dopunske datoteke u sistemu **aseestant**. Slova, brojevi i simboli treba da su jasni i ujednačeni, a dovoljne veličine da prilikom umanjivanja budu čitljivi. Slike treba da budu jasne i obeležene brojevima, onim redom kojim se navode u tekstu (**Sl. 1; Sl. 2** itd.). Ukoliko je slika već negde objavljena, obavezno citirati izvor.

Legende za ilustracije pisati na posebnom listu, koristeći arapske brojeve. Ukoliko se koriste simboli, strelice, brojevi ili slova za objašnjavanje pojedinog dela ilustracije, svaki pojedinačno treba objasniti u legendi. Za fotomikrografije navesti metod bojenja i podatak o uvećanju.

Skraćenice i akronimi

Skraćenice i akronimi u rukopisu treba da budu korišćeni na sledeći način: definisati skraćenice i akronime pri njihovom prvom pojavljivanju u tekstu i koristiti ih konzistentno kroz čitav tekst, tabele i slike; koristiti ih samo za termine koji se pominju više od tri puta u tekstu; da bi se olakšalo čitaocu, skraćenice i aktinome treba štedljivo koristiti.

Abecedni popis svih skraćenica i akronima sa objašnjenjima treba dostaviti pri predaji rukopisa.

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