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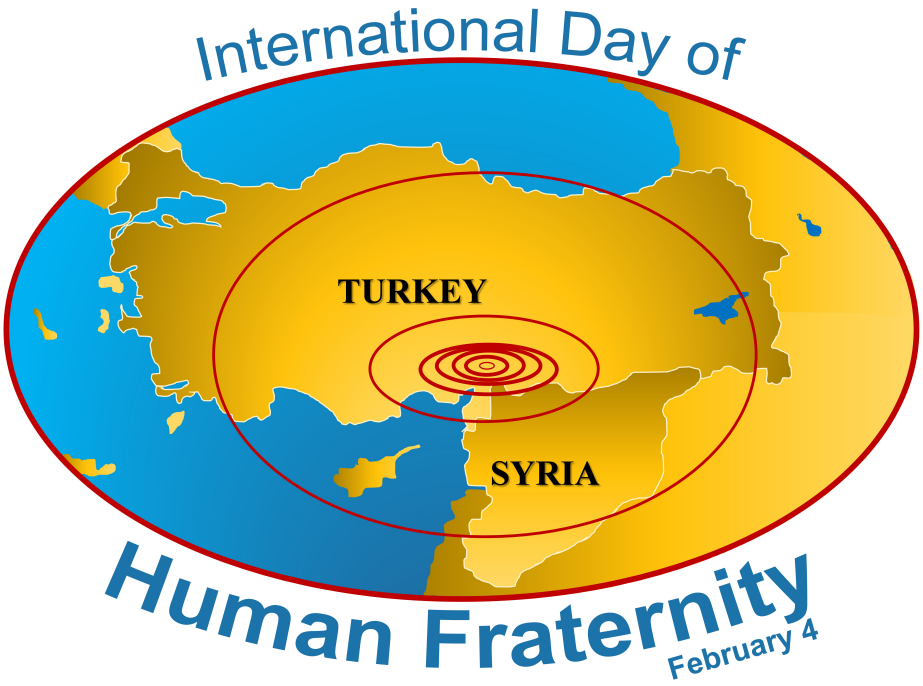

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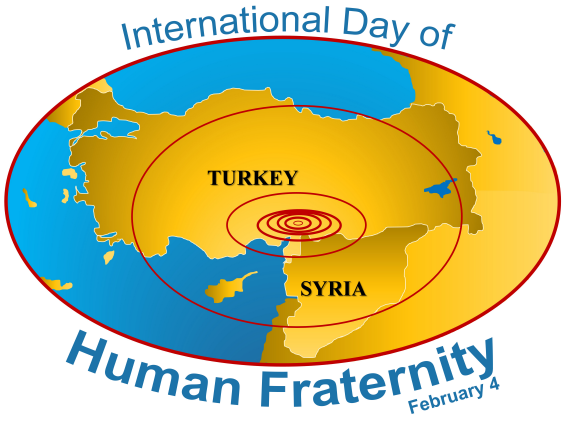

The United Nations General Assembly adopted a resolution declaring February 4 as the International Day of Human Fraternity to be celebrated annually from 2021.
The common values we share as human beings are especially important in days of great calamities that have befallen millions of people on our planet. One of the tragedies we are witnessing these days is the devastating earthquake in Turkey and Syria that occurred on February 6, 2023.
As the awareness of the scale of the tragedy and the number of dead and missing people grows, so does the need for every person to help the victims because we are all part of one humanity. We believe that the spirit of togetherness and the realization that they are not left to fend for themselves will give additional strength to the people in the earthquake-affected areas and help them recover faster.

Generalna skupština Ujedinjenih nacija usvojila je rezoluciju kojom se 4. februar proglašava Međunarodnim danom ljudskog bratstva, koji se obeležava svake godine počev od 2021. godine.
Zajedničke vrednosti koje delimo kao ljudska bića posebno su važne u danima velikih nesreća koje su zadesile milione ljudi na našoj planeti. Jedna od tragedija kojoj prisustvujemo ovih dana je razorni zemljotres u Turskoj i Siriji koji se dogodio 06. februara 2023. godine.
Kako raste svest o razmerama tragedije i broju poginulih i nestalih ljudi, raste i potreba svakog čoveka da pruži pomoć nastradalima, jer svi smo deo jednog čovečanstva. Verujemo da će duh zajedništva i spoznaja da nisu prepušteni sami sebi pružiti dodatnu snagu ljudima u zemljotresom pogođenim područjima i pomoći u njihovom bržem oporavku.

ORIGINAL ARTICLES

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C-reactive protein is a more valuable marker in predicting the severity of complications in measles-affected children compared to blood cell count-derived inflammatory indices

C-reaktivni protein je pouzdaniji pokazatelj od inflamacijskih indeksa izvedenih iz krvne slike u predviđanju teških komplikacija kod dece obolele od malih boginja

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Abstract

Background/Aim. Measles is a contagious disease with a good prognosis; however, severe complications may sometimes develop. C-reactive protein (CRP) and blood cells count-derived inflammatory indices – granulocyte-lymphocyte ratio (GLR), platelet to lymphocyte ratio (PLR), monocyte to lymphocyte ratio (MLR), mean platelet volume (MPV)/platelet count ratio (MPR), red blood cell distribution width (RDW), and MPV are the indicators related to the clinical outcome in various inflammatory diseases. The aim of the study was to analyze the values of CRP, blood cell count, GLR, PLR, MLR, MPR, RDW, and MPV in measles-affected children compared to healthy controls and between measles-affected children with complicated and severely complicated measles form. A particular aim of the paper was to assess the suitability of inflammatory-derived markers for predicting the severity of the disease. **Methods.** The study included 55 measles-affected children who developed complications (examination group), while the control group included 30 healthy children. The first peripheral blood count, obtained on the first hospitalization day (before treatment), was used for further analyses. **Results.** The white blood cells, lymphocytes, monocytes, and platelets count were significantly lower, while GLR, PLR, MPR, and CRP were significantly higher in measles-affected children ($p < 0.05$). In severely complicated measles form, significantly higher values of granulocytes, CRP, GLR, and PLR were documented, including lower lymphocytes ($p < 0.05$). A linear regression analysis showed that CRP was the only indicator with predictive significance for the severity of the course of measles. **Conclusion.** The blood cell count-derived inflammatory indices should not be crucial in assessing the severity of measles in children. CRP was the most valuable predictive factor for the development of the severe course of measles in measles-affected children.

Key words:
blood cells; blood platelets; child; c-reactive protein; leukocytes; measles; prognosis; severity of illness index.

Apstrakt

Uvod/Cilj. Male boginje (MB) su infektivno oboljenje koje u pojedinim slučajevima može dovesti do razvoja ozbiljnih komplkacija opasnih po život. Kao pouzdani pokazatelji kliničkog ishoda u mnogim inflamacijskim bolestima pokazali su se C-reaktivni protein (CRP) i indeksi inflamacije izvedeni iz krvne slike: odnos granulocita i limfocita (GLO), odnos trombocita i limfocita (TLO), odnos monocita i limfocita (MLO), odnos srednje zapremine trombocita (SZT) i broja trombocita (STO), varijacije u veličini u volumenu eritrocita (VVE) i SZT. Cilj rada bio je da se analiziraju vrednosti CRP-a, broja krvnih ćelija, GLO, TLO, MLO, STO, VVE, i SZT kod dece sa MB i uporede sa vrednostima kod zdrave dece, kao i da se ove vrednosti analiziraju i uporede između dece sa lakšim i teškim komplikacijama MB. Poseban cilj rada bio je da se proceni adekvatnost navedenih markera inflamacije za predviđanje težine MB. **Metode.** Ispitivanu grupu činilo je 55 dece obolele od MB, sa komplikacijama, a kontrolnu grupu činilo je 30 zdrave dece. U daljem istraživanju korišćen je prvi uzorak krvi, uzet prvog dana hospitalizacije (pre lečenja). **Rezultati.** Broj belih krvnih zrnaca, limfocita, monocita i trombocita bio je značajno niži, dok su vrednosti GLO, TLO, STO i CRP-a bile značajno više kod dece obolele od MB ($p < 0,05$). Kod dece sa težim komplikacijama MB zabeležene su značajno više vrednosti granulocita, CRP-a, GLO, TLO i niže vrednosti limfocita ($p < 0,05$). Metodom linearne regresije pokazano je da je

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vrednost CRP-a bila jedini pouzdan pokazatelj u prognozi nastanka teške forme MB. **Zaključak.** Broj krvnih ćelija i indeksi inflamacije izvedeni iz krvne slike ne bi trebalo da budu presudni u proceni ozbiljnosti kliničkog toka MB kod dece. CRP je pozdaniji pokazatelj u predviđanju razvoja težeg oblika bolesti kod dece obolele od MB.

Ključne reči:
krv, ćelije; trombociti; deca; c-reaktivni protein; leukociti; morbili; prognoza; bolest, indeks težine.

Introduction

Measles is an infectious disease caused by the highly contagious measles virus (MV). It is a preventable disease, and accelerated immunization against MV had a significant impact on reducing death caused by measles worldwide. However, measles still poses a public health problem. In 2018, more than 140,000 people died from measles, mostly children under 5 years of age [1]. From 2016 to March 2019, the European Region reported 114,682 measles cases [2].

As an infectious disease, measles generally has a good prognosis; however, severe complications may sometimes develop. The most common measles complications include pneumonia, croup, gastroenteritis, otitis media, conjunctivitis, and stomatitis. Rarely, uncommon complications such as encephalitis, myocarditis, pneumothorax, appendicitis, and subacute sclerosing panencephalitis may occur and can be very serious, life-treating, and require special treatment and care [3, 4].

Measles is a systemic inflammatory disease with general immune suppression which extends to more than two years after acute infection [5, 6]. The severe inflammatory process contributes to weak adaptive response (suppression of lymphocyte proliferation, altered cytokine profiles, immune modulation, and inhibition of hematopoiesis) and immune response imbalance [7–9].

Numerous inflammatory processes in the skin, respiratory mucosa, lung, conjunctivae, and liver are accompanied by the release of many pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, which can induce profound changes in C-reactive protein (CRP) synthesis and cytopoiesis of the hematic cells [5, 6, 10].

The level of CRP is a widely used marker of inflammation in clinical practice. Increased levels of pro-inflammatory cytokines (IL-6, IL-1β, and TNF-α), secreted mainly by macrophages and neutrophils, perform transcriptional induction of the CRP gene. CRP is mainly synthesized in hepatocytes but also by smooth muscle cells, macrophages, endothelial cells, lymphocytes, and adipocytes. CRP plays an important role in inflammatory processes during infection, including the complement pathway, apoptosis, phagocytosis, nitric oxide (NO) release, and the production of cytokines, particularly IL-6 and TNF-α [11].

These inflammation-induced changes are manifested in hematopoiesis and granulocyte, lymphocyte, and platelet (PLT) counts in the peripheral blood [7, 8]. The granulocyte count can reflect the inflammatory state in the body. The more severe inflammation, the higher the granulocyte count will be. At the same time, a more intensive inflammatory reaction will cause a lower lymphocyte count. The severity of inflammation reflects changes in the granulocyte-lymphocyte ratio (GLR). PLT to lymphocyte ratio (PLR) reflects changes in PLT and lymphocyte counts. It can demonstrate the severity of infectious diseases and the degree of thrombosis in in the body [12]. In clinical work, three laboratory parameters indicate PLT activation: the count of PLTs; mean PLT volume (MPV), the average size of PLTs, which reflects their production in the bone marrow; PLT cell distribution width (PDW), the index of inhomogeneity of the size of the PLTs. In healthy populations, MPV and PLT counts are in an inverse relationship. Therefore, reactive thrombocytosis is followed by lower values of MPV and PDW. Higher MPV is considered an indicator of PLT activation and aggregation. However, in an inflammation process, PLTs are quickly spared at the site of infection, and bone marrow accelerates the release of immature, larger PLTs [13, 14]. Inflammation and oxidative stress suppress erythrocyte maturation in the bone marrow and reduce the red blood cell (RBC) life span. As a consequence, large, premature RBCs (reticulocytes) are released into the circulation, and RBC distribution width (RDW) in the peripheral blood is elevated [15].

Recent studies have indicated that several inflammatory indices derived from blood cell count may be used in estimating the severity of inflammation as predictive markers in the diagnosis and prognosis of patients with inflammation (cardiovascular diseases, diabetes, malignancy) [16–18]. The novel inflammatory biomarkers include the following ratios: GLR, PLR, RDW, MPV, monocytes to lymphocytes (MLR), and MPV/PLTs count (MPR). These indices are inexpensive and easily calculated indicators of the systematic inflammatory response. Many studies have confirmed their usefulness in predicting sepsis in children [19], early onset neonatal sepsis [20], infective endocarditis [21], death risk in children with severe hand, foot, and mouth disease [22], and mortality in pediatric intensive care [23]. Furthermore, in other infectious diseases, these indices have the diagnostic and prognostic value in children with infectious diseases such as rotavirus-positive gastroenteritis [24], febrile seizures [25], Kawasaki disease [26], hepatitis A [27], otitis media [28], febrile urinary tract infection [29], and acute pyelonephritis [30].

While reviewing the literature, we found that Solmaz et al. [31] documented the utility of MPV only for determining inflammation in measles-affected children. Güzelçiçek and Demir [32] documented that only PLR was associated with the measles outcome in children.

The diagnostic and prognostic utility of CRP and GLR, PLR, MPV, MLR, MPR, and RDW has not been evaluated yet in measles-affected children with various severe forms of measles. The aim of the study was to analyze the values of CRP and GLR, PLR, MLR, MPR, RDW, and MPV in mea-

sles-affected children compared to healthy children and between two groups of measles-affected children, severely complicated and non-severely complicated. A particular aim of the paper was to assess the suitability of CRP and these blood cell count-derived inflammatory markers for predicting the severity of the disease.

Methods

During the outbreak of measles in Jablanica District, from October 2017 to July 2018, 110 children were affected. In the Pediatrics Department of the General Hospital Leskovac, 89 (80.9%) measles-affected children were clinically examined, and 55 (50%) were hospitalized and clinically treated. The criteria for hospitalization were the presence of measles complications and the parents' consent for hospital treatment. This retrospective, medical record-based study was conducted at the Pediatrics Department, General Hospital Leskovac, Serbia, and included 55 measles-affected, hospitalized children who developed complications and 30 healthy control children. The inclusion criteria for the study group were measles-affected children who developed complications. Exclusion criteria were measles-affected children without complications and children with chronic conditions (rheumatic, autoimmune, gastroenterological, hematological, malignant, nephrological, endocrinological, and neurological diseases). The control group included only healthy children. There were children for specialist outpatient pediatric examination in whom blood analyses were needed. These were the children who were referred to specialists due to suspicion of a disease that was excluded by diagnostic and clinical trials (anemia, short stature, impaired glucose, lipid metabolism, and hypothyroidism). Exclusion criteria for the control group were the presence of any infectious or chronic disease. Written informed consent was obtained from the parents of each child. The study was approved by the Ethics Committee of the General Hospital Leskovac (No 352/2 from January 20, 2021).

On admission to Pediatrics Department, clinical examination and venous puncture for laboratory analysis were performed. The diagnosis of measles was based on the World Health Organization criteria [3]. In cases of confirmed contact with the measles-affected and typical clinical manifestation (generalized erythematous/maculopapular rash, fever above 38 °C, cough, coryza, and conjunctivitis), measles was diagnosed. The diagnosis was made by a pediatrician and infectious disease specialist. Moreover, serological confirmation was performed in all cases at the Institute for Virology, Vaccines, and Serums "Torlak" in Belgrade. The detection of specific IgM antibodies in serum confirmed the diagnosis of measles.

Complications (diarrhea, dehydration, laryngitis, bronchitis, purulent conjunctivitis, and stomatitis) were determined by anamnestic data and physical examination. Otitis media was confirmed by an otoscopic examination. Pneumonia was confirmed by a chest radiograph with the presence of pulmonary infiltrates. The central nervous system was considered to be affected if lethargy, irritability, febrile seizures, disorientation, or other neurological deficits were present.

After diagnosing measles complications, the severity of complications and clinical conditions was assessed for every child. The clinical severity of measles was classified into two categories: complicated and severely complicated. The severely complicated group included children with convulsions, children who were lethargic or unconscious, chest indrawing with a respiratory rate of 60 breaths per min or more (oxygen saturation below 92% on room air), stridor in a calm child, severe dehydration, and severe malnutrition. The measles group with complications included children with rapid breathing (40 or more breaths per min for children older than 1 year or 50 or more breaths per minute for children under 1 year), moderate dehydration, laryngeal stridor only when a child was crying, mouth ulcers affecting the intake of food or fluids, pus draining from eyes, and acute otitis media.

Peripheral venous blood samples were collected in ethylenediaminetetraacetic acid tubes on the first hospitalization day. These results were chosen for further investigations. The complete blood count was analyzed on the Pentra ES 60 Horiba device. CRP was determined on Erba Mannheim XL 600 device.

The following laboratory data were noted: white blood cells (WBC), granulocyte, lymphocyte, and monocyte count and percentages, RBCs count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), RDW, and PLT count. GLR was calculated by dividing the absolute granulocyte count by the absolute lymphocyte count. PLR was calculated by dividing the absolute PLT count by the absolute lymphocyte count. MLR was calculated by dividing the absolute monocyte count by the absolute lymphocyte count. MPR was calculated by dividing the machine-calculated MPV by the PLT count.

Statistical analysis

The data were analyzed using descriptive statistical methods: frequency, percentage, mean ± standard deviation, and 95% confidence interval (CI) for Exp(B), depending on the type and variable distribution. Kolmogorov-Smirnov's test confirmed the deviation from the normality of data distribution. Relation between variables was measured using non-parametric Mann-Whitney *U* test and *t*-tests. The logistic regression (Enter model) was performed to determine the predictive importance of significant variables for the severely complicated measles form. Variables with statistical significance between non-severely complicated and severely complicated measles forms were included in a linear regression entry method. All analyses were conducted with SPSS version 17.0 (SPSS INC, NC). Statistical significance was set at $p < 0.05$.

Results

A total of 55 hospitalized measles-affected children were included in the study. The mean age of the measles group (n = 55) was 22.85 ± 23.94 months. The mean age of the non-severely complicated group and the severely com-

plicated group was 23.84 ± 26.02 and 20.65 ± 19.03 months, respectively. As shown in Table 1, there were no significant differences between the groups regarding age and sex ($p > 0.05$).

Measles-affected children had significantly higher values of WBC, percentage of granulocytes, PLT, MCV, GLR, PLR, MPR, and CRP compared to the control group ($p = 0.010$, $p = 0.001$, $p = 0.0001$, $p = 0.004$, $p = 0.0001$, $p = 0.022$, $p = 0.002$, $p = 0.0001$). Absolute counts and percentages of lymphocytes and monocytes were significantly lower in measles-affected children compared to the control group ($p < 0.005$) (Table 2).

The percentage of granulocytes was significantly higher ($p = 0.014$), while the number and percentage of lymphocytes were significantly lower ($p < 0.01$) in the severely complicated measles group. The values of inflammatory indices, GLR, PLR, and CRP were significantly higher in the severely complicated group compared to the non-severely complicated group ($p = 0.018$, $p = 0.012$, $p = 0.002$, respectively) (Table 3).

Additionally, variables with statistical significance between the non-severely complicated and severely complicated measles forms were included in the linear regression entry method. As a result, the value of CRP had a predictive effect on the development of the severe form of measles (Table 4).

Table 1

Age and gender of the patients

Variables	Control group	Measles group			*p*-value	
		total	NSC	SC	MG vs. CG	SC vs. NSC
Age (months) mean ± SD	24.9 ± 25.86	22.85 ± 23.94	23.84 ± 26.02	20.65 ± 19.03	0.740	0.913
Gender						
male, n (%)	14 (46.66)	27 (49.09)	22 (57.89)	5 (29.41)	0.9893	0.096
female, n (%)	16 (53.33)	28 (50.90)	16 (42.10)	12 (70.58)		

SD – standard deviation; NSC – non-severely complicated measles group; SC – severely complicated measles group; MG – measles group; CG – control group; n – number.

Table 2

Laboratory tests and blood cell count-derived inflammatory indices performed in measles-affected children and the control group

Parameters	Control group (n = 30)	Measles group (n = 55)	*p*-value	Reference range
WBC (10^9/L)	9.110 ± 2.803	7.560 ± 3.378	0.010*	6.0–16.0
RBC (10^{12}/L)	4.402 ± 0.449	4.631 ± 0.547	0.053	4–5
Hgb (g/L)	124.970 ± 16.243	113.580 ± 15.081	0.002*	109–138
Hct (%)	36.567 ± 4.427	34.553 ± 3.736	0.029*	28.8–39
MCV (fL)	81.437 ± 6.498	74.649 ± 11.461	0.004*	73.8–89.4
RDW (%)	12.587 ± 0.945	13.371 ± 2.992	0.167	11.9–16.2
PLT (10^9/L)	323.030 ± 84.272	249.710 ± 90.752	< 0.001*	150–450
MPV (fL)	8.253 ± 0.686	8.207 ± 1.144	0.841	6.9–11.3
Granulocytes (%)	40.547 ± 17.577	55.536 ± 18.728	0.001*	42–76
Granulocyte count (10^9/L)	3.481 ± 1.627	4.155 ± 2.684	0.400	1–8.5
Lymphocytes (%)	53.493 ± 16.751	39.818 ± 17.442	0.001*	11–49
Lymphocyte count (10^9/L)	5.111 ± 2.651	3.010 ± 1.903	< 0.001*	4–12
Monocytes (%)	5.640 ± 2.091	4.318 ± 2.636	0.005*	0–10
Monocyte count (10^9/L)	0.546 ± 0.310	0.336 ± 0.297	< 0.001*	0.3–1.0
GLR	0.724 ± 0.401	2.005 ± 1.800	< 0.001*	
MLR	0.129 ± 0.058	0.129 ± 0.099	0.424	
PLR	81.906 ± 51.737	114.949 ± 85.456	0.022*	
MPR	0.026 ± 0.009	0.038 ± 0.017	0.002*	
CRP (mg/L)	1.823 ± 1.329	15.320 ± 20.066	< 0.001*	0–5

WBC – white blood cell; RBC – red blood cell; Hgb – hemoglobin; Hct – hematocrit; MCV – mean corpuscular volume; RDW – red cell distribution width; PLT – platelet; MPV – mean platelet volume; GLR – granulocyte/lymphocyte ratio; MLR – monocyte/lymphocyte ratio; PLR – platelet/lymphocyte ratio; MPR – mean platelet volume/platelet count ratio; CRP – C-reactive protein. Values are expressed as mean ± standard deviation. Mann-Whitney *U* test.
***statistically significant difference.**

Table 3

Laboratory tests and cell count-derived inflammatory index performed in non-severely complicated and severely complicated measles form (MF) groups

Parameters	Non-severely complicated MF (n = 38)	Severely complicated MF (n = 17)	*p*-value	Reference range
WBC (10^9/L)	7.539 ± 2.8792	7.606 ± 4.3974	0.792	6.0–16.0
RBC (10^{12}/L)	4.5895 ± 0.58399	4.7241 ± 0.45699	0.591	4–5
Hgb (g/L)	113.21 ± 16.313	114.41 ± 12.294	0.870	109–138
Hct (%)	34.537 ± 4.0802	34.588 ± 2.9336	0.978	28.8–39
MCV (fL)	75.879 ± 9.5345	71.900 ± 14.8832	0.542	73.8–89.4
RDW (%)	13.855 ± 2.061	12.288 ± 4.3107	0.616	11.9–16.2
PLT (10^9/L)	254.21 ± 90.690	239.65 ± 92.849	0.466	150–450
MPV (fL)	8.355 ± 1.1332	7.876 ± 1.1311	0.211	6.9–11.3
Granulocytes (%)	51.211 ± 17.2446	65.206 ± 18.7687	0.014	42–76
Granulocyte count (10^9/L)	3.731 ± 1.7444	5.104 ± 3.9868	0.702	1–8.5
Lymphocytes (%)	43.745 ± 16.645	31.041 ± 16.3577	0.010*	11–49
Lymphocyte count (10^9/L)	3.380 ± 1.9636	2.181 ± 1.5000	0.014*	4–12
Monocytes (%)	4.597 ± 2.5008	3.694 ± 2.8964	0.101	0–10
Monocyte count (10^9/L)	0.375 ± 0.3187	0.248 ± 0.2262	0.093	0.3–1.0
GLR	1.5593 ± 1.289	3.0009 ± 2.36028	0.018*	
MLR	0.121110 ± 0.073	0.147006 ± 0.1421789	0.649	
PLR	100.7352 ± 78.568	146.7194 ± 93.91298	0.012*	
MPR	0.03782 ± 0.017	0.03724 ± 0.015092	0.750	
CRP (mg/L)	10.626 ± 16.222	25.812 ± 24.1045	0.002*	0–5

For abbreviations see under Table 2.

Table 4

Linear regression analysis of predictors for severe measles form

Variables	B	SE	Wald	df	Sig.	Exp(B)	95% CI for EXP(B)	
							Lower	Upper
Lymphocytes (%)	0.269	0.156	2.994	1	0.084	1.309	0.965	1.776
Lymphocyte (count)	-0.732	0.457	2.562	1	0.109	0.481	0.196	1.179
Granulocytes (%)	0.191	0.140	1.866	1	0.172	1.210	0.920	1.591
PLR	0.004	0.005	0.586	1	0.444	1.004	0.994	1.014
CRP	0.066	0.026	6.149	1	0.013*	1.068	1.014	1.125
GLR	0.347	0.408	0.726	1	0.394	1.415	0.637	3.147
Constant	-22.959	13.956	2.706	1	0.100	0.000		

PLR – platelet/lymphocyte ratio; CRP – C-reactive protein; GLR – granulocyte/lymphocyte ratio; CI – confidence interval.
***statistically significant difference.**

Discussion

In searching for parameters useful for predicting the measles course, it is necessary to know the immunological mechanism involved in the inflammatory process. That implies migration and infiltration of neutrophils and PLTs into the perivascular space, which regulates the inflammation process in tissue via the cytokine network. Neutrophils are the first effector cells at the site of inflammation. They are involved in the removal of extracellular pathogens and the activation and control of other immune cells, especially PLTs [5–7, 33]. Blood monocytes, as a part of the innate immune system, migrate into the inflamed tissue and differentiate into macrophages and dendritic cells [34]. Defective lymphoproliferation, increased activation and apoptosis of uninfected lymphocytes induce significant lymphopenia in MV infection. De Vries RD et al. [35] hypothesized that measles immune suppression is, in fact, a "numbers game". They considered that measles immune suppression is a result of immune cell depletion "which is masked by the rapid proliferation of MV-specific and bystander lymphocytes". Recently published above-mentioned studies confirmed a significantly higher prognostic value of blood cell count-derived inflammatory indices in accessing the severity of inflammation than the count of particular types of blood cells.

CRP is a well-known marker of inflammation widely used in clinical practice. Elevated CRP levels are typical for bacterial infections but may also be recorded in some viral infections [36, 37]. In our study, the values of CRP corresponded to the severity of the inflammation, and it was the only valuable predictor for severely complicated measles forms in measles-affected children. The MV is the initiator of the inflammatory process, which causes extensive epithelial damage. These epithelial lesions are a favorable environment for the development of bacterial infection in immunocompromised children. It causes complications such as pneumonia and croup. These conditions cause the severe form of measles and represent the most common complications in the group of children with serious complications in our study. Therefore, elevated CRP is probably a result of bacterial superinfection or severe viral infections in response to elevated pro-inflammatory cytokines IL-1, IL-6, and TNF-α. Griffin

DE et al. [38] documented higher values of CRP in measles patients with pneumonia, which remained elevated for a long time, while CRP values in MV meningitis and encephalitis remained normal. It is caused by a direct viral invasion of the brain or an abnormal immune response to CNS antigens.

Altered peripheral blood cell count was confirmed in our study, and the results indicated the participation of the white blood cell subset in the pathogenesis of measles. Previous research by Solmaz et al. [31] showed significantly lower values of leukocytes, neutrophils, and PLTs in measles-affected children without significant differences in blood-derived indices NLR (neutrophil-lymphocyte ratio) and PLR. A recently published study by Güzelçiçek and Demir [32] on measles-infected children in comparison with healthy controls has shown a lower number of WBC and neutrophils, MPV and NLR, and higher number of lymphocytes and CRP. Only PLR was associated with the outcome of disease. A study by Kim et al. [39] has shown that the measles patients had lower leukocytes, neutrophils, and lymphocyte counts than the healthy group. In our study, we demonstrated significantly lower WBC, lymphocyte, monocyte, and PLT counts and a high granulocyte count in the measles group. A significantly higher count of granulocytes and the value of GLR confirm their participation in the measles pathogenesis. Defective lymphoproliferation and apoptosis of uninfected lymphocytes induce significant lymphopenia, which correlates with the severity of the disease [8, 9]. Changes in the total leukocyte pool present in our respondents, especially in granulocyte and lymphocyte counts, affected the values of GLR as well. The changes in GLR values were associated with more severe forms of measles. Nonetheless, the values of granulocytes, lymphocytes, and GLR were not valuable predictors of severely complicated measles forms in measles-affected children. This “numbers game” may be the reason why lymphocytes do not significantly change their numerical relationship with neutrophils, monocytes, and PLTs.

Increased levels of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 are responsible for reactive thrombocytosis and the severe course of measles. Activated PLTs release IL-1α, IL-1β, TGF-β1, macrophage inflammatory protein, platelet-derived growth factor. In addition, they express the surface molecules (E selectin, P selectin) involved in the chemotaxis of neutrophils, T-lymphocytes, natural killer cells, and macrophages [40, 41]. In inflammation, the bone marrow produces immature, larger PLTs followed by lower values of MPV. In this study, only the values of PLR were associated with a more severe measles form and followed the severity of the inflammatory process. Leukocyte shifts during the immune response (neutrophilia, lymphopenia) change the value of PLR. However, PLR was not a suitable predictive factor for severely complicated measles forms. A recent study has suggested that PLT activation may be a consequence of enhanced bacterial lipopolysaccharide in circulation. As measles is a viral infection, this may be the reason for a weaker activation of PLTs. CRP is a much more sensitive marker of inflammation than an indicator of PLT activation [41]. In the study of Solmaz et al. [31], the level of MPV was proved to be lower in measles-affected children. We demonstrated only a significantly lower PLT count in measles-affected children and higher MPR, which did not differ significantly in severe measles forms. Viruses can infect megakaryocytes, which leads to apoptosis of megakaryocytes, decreased production of PLTs, difficult maturation, and decreased expression of thrombopoietin receptor [40–42]. In our study, MPV was similar in measles-affected children and healthy controls, and in severe form vs. non-severe measles form. Data about usefulness in other inflammatory diseases in pediatrics are divergent. Nonetheless, PLR values differed significantly between the examined groups. In response to some viruses, PLTs can be activated. All these processes contribute to the increase in PLT consumption and removal and lead to thrombocytopenia [40, 42]. However, virus-induced PLT activation modulates the shape of immune responses, which may be one reason for the long-term immunosuppression present in measles-infected children [41].

The RDW is recognized as a biomarker of subclinical infection. The results of some studies showed that RDW positively correlated with inflammatory markers such as erythrocyte sedimentation and CRP levels. However, results of studies in the pediatric population are various, and RDW did not show as good a prognostic marker as in the adult population [43–45]. In our study, RDW did not show any significant relationship with inflammation severity.

A retrospective study and a small study group (n = 55), especially a small number of children with a seriously complicated form of measles (n = 17), limit the value of the results. Another limiting factor may be the time from the onset of symptoms to hospital admission and blood sampling. Some children had received antipyretics and antibiotics due to fever before hospital admission. Changes in the population of WBC and PLTs reflect the body’s response to the inflammatory and infectious process that can be modified by pharmacological treatment. Hence, data about the usefulness of these inflammatory biomarkers are various and remind us that laboratory results should be interpreted following the clinical conditions of every particular child. It is necessary to determine the cut-off values of CRP and tested ratios in measles-affected children who require hospital admission.

Conclusion

The results demonstrated that CRP was the most valuable predictor of severely complicated measles forms in measles-affected children. The blood cell count-derived inflammatory indices (GLR, MLR, PLR, and MPR) are not reliable predictive factors. The significance of the CRP value and blood-derived inflammatory parameters for predicting the severity of measles should be further examined in other multicenter studies with a larger study group.

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Analgesic protocol for procedural pain treatment of second-degree burns in children

Analgetski protokol u ublažavanju proceduralnog bola kod dece sa opekotinama drugog stepena

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Abstract

Background/Aim. Children with burns are submitted to multiple painful and anxiety-related procedures during the change of wound dressing, treatment, and rehabilitation. The objective of analgesic treatments for procedural pain is the safe and efficient management of pain and emotional stress, which requires a careful, balanced, and systematic approach. The aim of this study was to determine the effectiveness of analgesic and/or local anesthetic in relieving the intensity of procedural pain. **Methods**. The study included 120 pediatric patients with second-degree burns who were allocated into four groups of 30 children (control group, groups I, II, and III). During the change of wound dressings, children in the control group did not receive any analgesics, while in the remaining three groups, 30 minutes prior to the change of wound dressing, oral nonsteroidal anti-inflammatory drug (group I), local anesthetic (group II), or both medications (group III) were administered. **Results.** The average visual analog scale (VAS) score for assessing pain was statistically significantly higher in the control group on all tested days compared with children in the other three treated groups. On the first test day (24 hrs after sustaining the burn injuries), all children had high VAS scores, and according to the receiver operating characteristics (ROC) analysis, the boundary value was 89.50/100. There was a remarkable difference in the VAS score between the groups on the fifth day of dressing change with the boundary value of 57.50/100 and on the seventh day when the boundary value was 43.50/100. Children who experienced the lowest intensity pain during dressing changes of burn wounds for all test days were those from the group who received both systemic analgesic and local anesthetic. **Conclusion.** The study confirmed the importance of introducing the complex polymodal protocol in treating procedural pain in second-degree burns. The protocol should include analgesics as well as anesthetics since they both contribute to achieving the best results in pain reduction and treatment outcomes.

Key words:
analgesics; anesthetics, local; burns; child; pain measurement; pain, procedural.

Apstrakt

Uvod/Cilj. Deca sa opekotinama su tokom previjanja, nege i rehabilitacije podvrgnuta višestrukim, bolnim procedurama koje izazivaju anksioznost. Primena analgetika za ublažavanje proceduralnog bola, ima za cilj bezbedno i efikasno upravljanje bolom i emocionalnim stresom, što zahteva pažljiv, balansiran i sistematičan pristup. Cilj rada bio je da se utvrdi u kojoj meri primena analgetika i/ili lokalnog anestetika ima uticaj na smanjenje intenziteta proceduralnog bola. **Metode.** U studiju je uključeno 120 pacijenata dečijeg uzrasta sa opekotinama drugog stepena koji su razvrstani u četiri grupe od po 30 dece (kontrolnu grupu i grupe I, II i III). Tokom previjanja, deca iz kontrolne grupe nisu primala analgetike, a deca u preostalim grupama su 30 minuta pre previjanja primali: nesteroidni antiinflamatorni lek, oralno (grupa I), lokalni anestetik (grupa II) ili oba leka (grupa III). **Rezultati.** Prosečni skor vizuelno analogne skale (VAS) za procenu intenziteta bola bio je statistički značajno veći u kontrolnoj grupi, tokom svih ispitivanih dana, u odnosu na preostale tri grupe dece koje su primile analgetike. Prvog dana (24 časa nakon zadobijanja opekotina), sva deca su imala visoke vrednosti VAS skora i prema *receiver operating characteristics* (ROC) analizi granična vrednost bila je 89,50/100. Vidna razlika između grupa u pogledu vrednosti VAS skora uočena je petog dana previjanja, sa graničnom vrednosti od 57,50/100 i sedmog dana kada je

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granična vrednost iznosila 43,50/100. Najmanji intenzitet bola prilikom previjanja, tokom svih ispitivanih dana, prijavljivala su deca u grupi koja je primila i sistemski analgetik i lokalni anestetik. **Zaključak.** Istraživanje je pokazalo značaj uvođenja složenog polimodalnog protokola u lečenju proceduralnog bola prilikom previjanja opekotina parcijalne debljine kože. Najbolji rezultati u smanjenju intenziteta bola i izlečenju postižu se primenom protokola koji uključuje i sistemsku analgeziju i lokalnu anesteziju.

Ključne reči:
analgetici; anestetici, lokalni; opekotine; deca; bol, merenje; bol, proceduralni.

Introduction

Burns continue to represent an important medical, social, and economic issue in modern society. The consequences of burn injuries are especially harsh in children since the functional, aesthetic, and psychological sequels are much more severe than in adults. Burns are experienced as traumatic events related to high stress and anxiety levels. Pain is an unpleasant subjective experience with sensory, emotional, and behavioral components [1–4].

Patients with burns are subjected to high levels of "expecting" pain before wound dressing, which is repeated daily and increases the patient's perception of pain. This anticipated treatment-related distress leads to an increased subjective perception of pain, which in turn reinforces the anxiety experienced by patients as a feedback loop. That explains the occurrence of intensified pain in burn victims during their hospitalization and follow-up [1, 5].

Nowadays, pain management in burn patients encompasses a wide spectrum of pharmacological and non-pharmacological treatment methods. Opioids, non-opioids [non-steroidal anti-inflammatory drugs (NSAIDs), acetaminophen, and topical lidocaine], antidepressants, anxiolytics, sedatives, alpha-adrenergic agonists, and others are used as pharmacological treatment. On the other hand, various adjuvant techniques (psychotherapy, distraction, music, virtual reality, massage, and others) are used as a non-pharmacological treatment to meet the patient's needs [1–3]. In the outpatient setting, pain relief is most commonly managed by NSAIDs [5, 6]. Despite numerous options for managing procedural pain, especially in the pediatric population, the lack of implementing pain relief techniques or their inadequate implementation is still quite common [7, 8].

The aim of this study was to evaluate whether the use of analgesics and/or local anesthetics had an effect on relieving the intensity of procedural pain in children.

Methods

In this study, 120 children were treated for burns for two years at the Clinic for Pediatric Surgery, Institute for Child and Youth Health Care of Vojvodina in Novi Sad, Serbia.

Patients included in the study were selected randomly and were divided into four equal groups of 30 children alternatively.

Inclusion study criteria were as follows: children of both genders aged 6 to 15 years with second-degree (IIa – partial thickness) burns which covered less than 10% of total body surface area located on the body and extremities (excluding the face, genitalia, and hands). After a detailed explanation of the methodology to the parents, written consent for the study was obtained.

Exclusion criteria were the following: burns on the face, genitalia, and hands; children who have taken antibiotics for any reason at least two weeks before hospitalization; all children with chronic illnesses, especially of the liver and kidneys; patients with previous diseases at least one month before being admitted to hospital; all patients treated with immunosuppressive therapy; patients with congenital anomalies; patients with psychomotor disturbances; patients with multiple injuries and traumas; patients with signs of wound infection during the study; patients without written consent for participation in this study.

Common clinical practice in the treatment of deep dermal and subdermal burns includes pain treatment, while minor burns (superficial dermal burns – level IIa, which do not require surgical treatment) are usually treated with daily dressing changes but without planned psychological preparation or analgesics. The dressing changes procedure consists of removing the dressing, washing the wound, applying the medication (silver sulfadiazine), and placing a new dressing. Since this type of treatment is associated with repeated painful experiences of different intensities and durations, we investigated to which extent the use of analgesics as standard procedure for dressing changes of level IIa burns (superficial dermal burns) had an impact on the patient's perception of pain during treatment and healing process.

The following medications were used in the study:

1. Ibuprofen (Brufen®, oral suspension 100 mg/5 mL, 100 mL, Galenika a.d.), an NSAID used for systemic analgesia.
2. Xylocaine® gel 5%, 30 g, galenic formulation, Pharmacy Belgrade, used as a topical anesthetic.

This prospective study was conducted at the Institute for Child and Youth Health Care during the two years from 2012 to 2014 and was approved by its Ethics Committee (Decision No 403-6).

All patients received standard primary surgical treatment of the burn upon obtaining the burns (day zero), and an absorptive bandage was applied.

Local treatment of the burn began 24 hrs after the injury, and afterward, the children were randomly divided and assigned to one of the following groups: a control group and groups I, II, and III.

The control group did not receive any analgesics before the change of wound dressing. Only regular distraction methods, such as talking, singing, or cartoon playing, were applied. The wound care started with toilette and debridement, and silver sulfadiazine was applied to the wound (a common manner of dressing minor burns).

Group I – the patients were treated with a 30 mg/kg dose of ibuprofen, and thirty minutes later, the wound was dressed, and silver sulfadiazine was applied.

Group II – when dressings were removed, Xylocaine® gel was applied to the burn, and the wound was wrapped; after thirty minutes, the wound was cleaned, silver sulfadiazine was applied, and the wound was then redressed.

Group III – thirty minutes prior to dressing, ibuprofen was administered; thirty minutes later, dressings were removed, and Xylocaine® gel was applied to the wound; thirty more minutes later, the wound was cleaned, and silver sulfadiazine was applied along with bandages.

The intensity of pain was measured by a visual analog scale (VAS) score (0–100), in which 0 indicated the absence of pain, while value 100 was explained as the worst possible pain. The pain was measured on the first, third, fifth, and seventh day after obtaining burns during the change of wound dressing. VAS values less than 30 were considered mild pain; VAS values ranging from 30–70 denoted moderate pain intensity; VAS values estimated at higher than 70 were considered severe pain.

Statistical Data Analysis

Descriptive statistics determined the average value, standard deviation (SD), or absolute frequency of occurrence with the corresponding percentages. The difference in percentages was tested using the Chi-squared (χ^2) test. The normality of distribution was determined using the Shapiro-Wilk test. Pain intensity in relation to the treatment group for each day of dressing changes was analyzed by one-way analysis of variance (ANOVA), Schaeffer's test. Key points for the first, third, fifth, and seventh day were determined based on receiver operating characteristic (ROC) curve analysis. Linear regression analysis was used to estimate the effects of therapy, age, and gender on the VAS score. All analyses were done using SPSS Statistics 23.0 (IBM, Chicago, USA), and the statistical significance was assessed at the level of p less than 0.05.

Results

Results of the regression analysis showed that the combined effects of the treatment method, gender, and age increased with the increasing time after burn injury (Table 1). There were no significant differences regarding gender and age. Only the treatment method had a significant effect on all examined days.

On the first day, during dressing changes, the average pain values were very high for all the children in all the groups (Table 2). Children from the control group reported pain significantly ($p < 0.05$) more intensively than children from groups I and III, while this difference between groups I and III was not significant. The pain was significantly ($p < 0.001$) less intensive after analgesic application on the third, fifth, and seventh day after burn injury in all the groups compared to the control. Children who experienced the intensity of pain the least during dressing changes after burn injury belonged to group III during the whole observed period.

Further results of the repeated measures analysis showed that VAS score values significantly decreased ($p < 0.001$) in all analyzed groups as time progressed (Table 3). On the first and the third day during dressing changes, all children from the control group had a VAS score higher than 70, which indicated severe pain, while after the fifth day, only 40% of children had severe pain, and after the seventh day all children in this group had moderate pain. In the groups of treated children, severe pain was noted in 80 out of 90 (88.9%) children on the first day and 66 out of 90 (73.3%) children on the third day. However, the pain significantly decreased on the fifth examined day, when only 6 out of 90 (6.7%) children reported severe pain. No child from the control group had a VAS score less than 30 (mild pain) during all examined days, while in all treated groups

Table 1

Effects of group, gender, and age on the visual analog scale scores during wound dressing changes

Time after burn injury (day)	Regression analysis						r
	group		gender		age		
	beta	p-value	beta	p-value	beta	p-value	
First	-0.204	**0.027**	0.041	0.651	0.058	0.527	0.214
Third	-0.293	**0.001**	0.050	0.573	0.002	0.980	0.295
Fifth	-0.449	**0.000**	0.138	0.095	0.069	0.403	0.470
Seventh	-0.508	**0.000**	0.052	0.513	-0.116	0.147	0.521

***p* – significance; *r* – multiple correlation coefficient. Bolded values are statistically significant.**

Table 2

Average visual analog scale score during wound dressing changes in the study groups

Time after burn injury (day)	Control	Group I	Group II	Group III
First	93.00 ± 5.43*	83.60 ± 16.16	89.47 ± 10.88	93.00 ± 5.43
Third	87.90 ± 7.04***	76.67 ± 15.92	79.83 ± 12.72	75.17 ± 13.61
Fifth	66.73 ± 10.33***	46.07 ± 13.52	52.60 ± 15.17	44.47 ± 10.78
Seventh	51.57 ± 7.53***	34.53 ± 14.75	30.50 ± 10.95	31.77 ± 11.12

Control – group without analgesic treatment; Group I – group treated with ibuprofen; Group II – group treated with Xylocaine® gel; Group III – group treated with ibuprofen + Xylocaine® gel.

Results are expressed as mean ± standard error. Statistical significance: *$p < 0.05$ compared to group II and III; ***$p < 0.001$ compared to group I, II, and III (One-way analysis of variance, Schaeffer's test).

(I, II, and III), 6 out of 90 (6.7%) patients on the fifth day and 43 out of 90 (47.8%) patients on the seventh day experienced mild pain.

The results of the χ^2 test showed a statistically significant difference in the intensity of pain between the groups with and without treatment on the third ($\chi^2 = 10.000$, df = 2, $p = 0.007$), fifth ($\chi^2 = 20.667$, df = 2, $p = 0.000$), and seventh day ($\chi^2 = 23.158$, df = 2, $p = 0.000$) after a burn injury.

The ROC analysis of the VAS score for 90 children who received analgesic treatment and thirty children without it showed that on the first day during bandaging, sensitivity was 0.800, and specificity was 0.544. The area under the ROC curve (AUC) of the first day showed a bad separation of children with applied treatment and children without it. All children had high VAS score values, and the boundary value was 89.50 [area under curve (AUC) = 0.656, 95% confidence interval (CI) = 0.558–0.753]. On the third day, there was acceptable separation (AUC = 0.750, 95% CI = 0.660–0.841). On the fifth day, separation was excellent (AUC = 0.863, 95% CI = 0.798–0.927), with a boundary value of 57.5. The results obtained on the seventh day after the burn injury showed exceptional separation during dressing changes. The boundary value was 43.50 (AUC = 0.900, 95% CI = 0.846–0.954) (Figure 1, Table 4).

Table 3

Distribution of children with and without treatment who experienced mild, moderate, or severe pain according to average visual analog scale score

Time (day)	Pain intensity	Group	
		without treatment (n=30)	with treatment (n=90)
First	Mild		1 (1.1)
	Moderate		9 (10)
	Severe	30 (100)	80 (88.9)
Third	Mild		1 (1.1)
	Moderate		23 (25.6)
	Severe	30 (100)	66 (73.3)
Fifth	Mild		6 (6.7)
	Moderate	18 (60)	78 (86.7)
	Severe	12 (40)	6 (6.7)
Seventh	Mild		43 (47.8)
	Moderate	30 (100)	46 (51.1)
	Severe		1 (1.1)

The results are given as n (%).

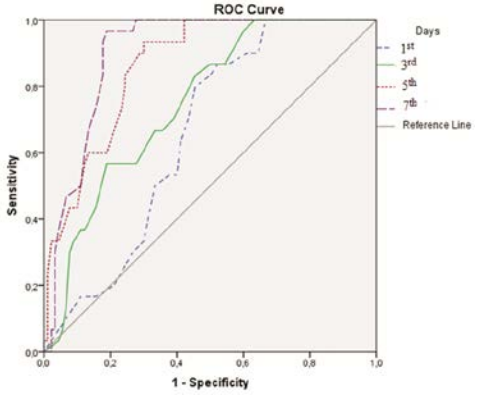

Fig. 1 – Receiver operating characteristic (ROC) curve shows the correlation of visual analog scale scores on the first, third, fifth, and seventh day after burn injury during wound dressing changes between patients with and without analgesics.

Table 4

Receiver operating characteristic analysis of visual analog scale scores during wound dressing changes with applied analgesic treatment

Parameter	Time after burn injury (day)			
	first	third	fifth	seventh
AUC	0.656	0.750	0.863	0.900
SE	0.050	0.046	0.033	0.027
Significance	0.011	0.000	0.000	0.000
95% CI	0.558–0.753	0.660–0.841	0.798–0.927	0.846–0.954
Cut-off value	89.50	84.00	57.50	43.50
Sensitivity	0.800	0.667	0.833	0.933
Specificity	0.544	0.667	0.756	0.822

AUC – area under curve; SE – standard error; CI – confidence interval.

Discussion

The study was conducted to assess the effectiveness of analgesic therapy for procedural pain in patients with superficial dermal burns. Children included in the study were 6 to 15 years old. A numerical VAS score was used to evaluate the intensity of pain. Literature supports the use of the VAS scale as it is simple, easy to use, reliable, and appropriate for this age group [8–10].

Since some studies emphasized the significance of gender and age on the perception of pain [11, 12], we have analyzed whether these variables had an impact on pain perception in our study. When observing gender differences, the results we obtained showed that during dressing changes, girls had a higher VAS score than boys, but the difference was not statistically significant. Van der Heijden et al. [1], Shahi et al. [5], and Khan et al. [13] examined the pediatric population exclusively in their studies and came to a similar conclusion, where no difference in the perception of pain regarding gender was noticed. When taking into account that some studies (Sorge and Strath [11], Mogil [12]) highlight the significance of physiological and psychosocial differences between genders in the adults' perception of pain, it is clear that this difference is subtler or nonexistent for children, which explains our results.

In terms of age, in our study, when all patients are considered as unique specimens (disregarding the group membership), younger children are found to experience a higher intensity of pain during dressing changes compared to older children, but the difference is not significant as well. The study conducted in South Africa reported a significantly higher intensity of pain perception in younger children. In this study [1], parents were not allowed to be with the children during dressing changes, while our practice is to encourage parents' presence during treatment. Another study measuring the levels of pain and distress in children with burns after dressing changes revealed no statistical significance concerning age. All the patients in this study reunited with their parents after the treatment when the measurement was conducted [2].

The underestimation of analgesic needs in children is quite widespread despite common knowledge of its negative effects on the physiologic (delayed wound healing, reduced mobility, hyperalgesia) and psychologic (anxiety, posttraumatic stress, depression) status of patients [3, 5, 14]. Even though experts stress the importance of analgesia for burn patients, many difficulties are encountered in providing pain management. Pain assessment in the pediatric population is particularly challenging due to the difficulty in differentiating pain from anxiety, hunger, or fear. Inadequate training of the staff, lack of knowledge concerning the safety and efficiency of analgesia, and established clinical malpractices are other important issues. The literature unanimously suggests the introduction of polymodal analgesic protocols to overcome the neglected pain [14–16].

We have chosen to investigate the effects of Xylocaine® gel and ibuprofen, which are the most frequently used treatment options for pain control in minor burns. Xylocaine® gel, cream, spray, or patch is widely employed as a topical anesthetic with effective pain control and rarely causes side effects [17, 19]. Ibuprofen is a commonly prescribed medication for burn victims. As a cyclooxygenase inhibitor, it strongly inhibits the pro-inflammatory interleukin-1 beta in cerebrospinal fluid and brain structures, resulting in a hypersensitivity decrease [7, 13, 20, 21]. Although opioid analgesics are mostly used in pain therapy for burns, we have left them out of the study. The main reason for doing so is the fact that only superficial dermal burns treated in outpatient conditions were included in the study, and in such situations, opioids are avoided due to possible negative side effects, such as respiratory depression, physical dependence, or tolerance.

While observing the intensity of pain, a significant decrease in its values could be noticed in all the groups from the first to the seventh day of dressing changes, when an epithelialization of the burn wound would gradually occur, and the burned area would become smaller. Similarly, Resch et al. [22] recorded a continuous decrease in pain intensity in the course of dressing changes in all the participants during the period they observed.

Children who did not receive any therapy during dressing changes (the control group) had a significantly higher VAS score than all the children in other groups. The only exception was on the first day when the VAS score in the control group was not significantly higher in comparison to the group of children treated with local anesthetic (group II). Better results in terms of analgesia were achieved in the groups treated with ibuprofen (groups I and III) compared to the group treated with a local anesthetic (group II). These results may be due to both the anti-inflammatory and analgesic effects of ibuprofen [20].

Van der Heijden et al. [1] divide different phases of wound care into four actions: removing the bandages, washing the wound, applying the wound care medication, and placing a new bandage. This classification into separate phases of dressing change may explain the higher intensity of pain in group II. In this group, the anesthetic was applied after the removal of the bandages; thus, the initial painful stimuli could not be inhibited.

The presence of intense pain during dressing changes indicates a need for the applied analgesic regime, and the best results in our study were achieved by combining local anesthetic and oral analgesic. There are suggestions for the potential use of opioid analgesics, especially in the first few days after injury [3].

Although the literature suggests that pain in minor burns could be successfully treated with either local anesthetic (lidocaine) or oral analgesic (NSAID) [6, 17], our experience suggests that the combination of those two offers better results in terms of patient satisfaction and overall treatment outcome.

Conclusion

The results of our study show that gender and age are not playing a significant role in pain perception, so they should not be used as indicators for predicting pain intensity

in the pediatric population. The importance of complex polymodal protocol introduced in the treatment of minor burns in children was confirmed. The protocol should include analgesics as well as local anesthetics since they both contribute to achieving a satisfactory treatment outcome. To improve clinical practice, continuing research is essential to explore different pharmacological and non-pharmacological approaches in dealing with procedural pain in burn patients.

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Altered high-density lipoprotein particle structure and antioxidant capacity in preeclampsia

Izmenjena struktura i antioksidativni kapacitet lipoproteinskih čestica visoke gustine u preeklampsiji

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Abstract

Background/Aim. One of the complications that can occur during pregnancy is the development of preeclampsia (PE). The main characteristics of this condition are high blood pressure and very often signs of kidney damage or other organ damage. The condition affects 5–7% of all pregnant women and is one of the main factors of maternal and perinatal morbidity and mortality worldwide. The aim of this study was to investigate the structural and functional modifications of high-density lipoprotein (HDL) particles during high-risk pregnancies (HRP) for PE development. **Methods.** The longitudinal prospective study included a total of 91 pregnant women with a HRP for developing PE. Out of this total number, 71 women did not develop PE until delivery, and this group was designated as the group without PE (WPE). The rest of the 20 HRP women developed PE before delivery and were designated as the PE group. The blood was sampled toward the end of each trimester and before the delivery. The distribution of HDL particles was determined by the vertical 3–31% polyacrylamide gradient gel electrophoresis method. The antioxidative capacity of HDL particles was measured by the activity of the HDL-associated enzyme – paraoxonase 1 (PON1). PON1 activity was determined by the method of kinetic spectrophotometry from serum samples. **Results.** The results have shown that the proportions of HDL_{2b} particles significantly increased in the 2nd trimester ($p < 0.05$) and remained increased until the end of pregnancy in the WPE group. PON1 activity was significantly higher in the 3rd trimester ($p < 0.05$) of the WPE group. In the PE group, we found that the proportions of HDL_{3a} particles significantly decreased in the 2nd trimester ($p < 0.05$) and remained decreased until the end of pregnancy. PON1 activity has not changed in the PE group during pregnancy. **Conclusion.** Dyslipidemia in pregnancy could be associated with different modifications of HDL particles. The adaptive pregnancy mechanisms expressed as a functional modification of HDL particles in pregnant women who develop PE seem inadequate and, therefore, lose their atheroprotective role.

Key words:
antioxidants; lipoproteins, hdl; preeclampsia; pregnancy, complications.

Apstrakt

Uvod/Cilj. Jedna od komplikacija koja se može javiti tokom trudnoće je razvoj preeklampsije (PE). Glavne karakteristike ovog stanja su visok krvni pritisak i vrlo često znaci oštećenja bubrega ili drugih organa. Ovo stanje pogađa 5–7% svih trudnica i jedan je od glavnih faktora morbiditeta i mortaliteta trudnica i fetusa ili novorođenčadi. Cilj ovog rada bio je ispitivanje strukturnih i funkcionalnih modifikacija lipoproteinskih čestica visoke gustine (*high-density lipoprotein* – HDL) u trudnoćama sa visokim rizikom (TVR) za razvoj PE. **Metode.** U longitudinalnoj prospektivnoj studiji učestvovala je ukupno 91 trudnica sa TVR od razvoja PE. Od ukupnog broja trudnica, kod njih 71, PE se nije razvila do kraja trudnoće i ova grupa je označena kao grupa bez PE (BPE). Kod preostalih 20 žena sa TVR se razvila PE do porođaja, i one su svrstane u grupu PE. Krv je uzimana za analizu na kraju svakog trimestra i pred porođaj. Raspodela HDL čestica je određivana metodom vertikalne elektroforeze u 3–31% gradijentu poliakrilamidnog gela. Antioksidativni kapacitet HDL čestica je određivan na osnovu aktivnosti enzima paraoksonaze 1 (PON1) vezanog za HDL. Aktivnost PON1 u serumu je određivana metodom kinetičke spektrofotometrije. **Rezultati.** Rezultati su pokazali da se udeo HDL_{2b} čestica značajno povećao u drugom trimestru ($p < 0,05$) i ostao je povećan do kraja trudnoće u grupi BPE. Aktivnost PON1 bila je značajno veća u trećem

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trimestru ($p < 0,05$) kod ove grupe trudnica. Udeo HDL_{3a} čestica se značajno smanjio u drugom trimestru u PE grupi trudnica ($p < 0,05$) i ostao je snižen do kraja trudnoće. Aktivnost PON1 enzima u PE gripi se nije menjala u toku trudnoće. **Zaključak**. Dislipidemija u trudnoći može biti posledica različitih modifikacija HDL čestica. Strukturne i funkcionalne modifikacije HDL čestica, kao jedan od adaptivnih mehanizama, kod trudnica kod kojih se razvila PE, nisu adekvatne i kao takve gube svoju ateroprotektivnu ulogu.

Ključne reči:
antioksidansi; lipoproteini hdl; preeklampsija; trudnoća, komplikacije.

Introduction

Preeclampsia (PE) is defined as a hypertensive disorder of pregnancy accompanied by proteinuria or some other organ damage, and it is recognized as one of the most severe pregnancy complications [1]. With an estimated incidence of 5%, PE is a leading cause of maternal and perinatal morbidity and mortality worldwide [2]. The main pathophysiological feature of PE is the insufficient remodeling of the spiral artery, leading to placental ischemia and creating an imbalance between anti-angiogenic and pro-angiogenic factors, finally inducing maternal endothelial dysfunction [3]. Despite a lot of research in this field, there are still major doubts regarding the prediction, monitoring, and therapy of this pregnancy complication. PE shares many risk factors with cardiovascular (CV) disease (CVD), such as obesity and hypertension. Thus, the role of the altered lipid profile in the pathogenesis of endothelial dysfunction in PE has become an important area of research during the last several decades [4]. Normal pregnancy is followed by metabolic changes, especially increased levels of plasma lipids, oxidative stress, and inflammation, as well as impaired coagulation processes. However, in PE, all those changes are much more pronounced [5, 6]. The most common lipid abnormalities in PE are hypertriglyceridemia with increased circulating free fatty acids, lower high-density lipoprotein (HDL) cholesterol (HDL-C) concentration, and a higher proportion of small dense (sd) low-density lipoprotein (LDL) particles (sdLDL) [4].

It has been recognized that PE and CVD share common risk factors, diabetes mellitus (DM), obesity, hypertension, and renal disease. In addition, PE could be associated with an increased risk of CVD development later in life [4, 7]. On the other hand, the role of HDL particle distribution, structure, and function in the CVD risk assessment has been studied extensively during the last few years. Surprisingly, data about this issue in PE is quite limited. HDL particles show diversity in their structure, composition, and functionality, including their different roles in lipid transport, antioxidative capacity, inflammation, and hemostasis [8]. The newest concept in understanding this lipoprotein indicates that HDL composition and functionality, much more than HDL-C concentrations, qualify the multipotent antiatherogenic role of this lipoprotein [9–11]. Kontush [11] highlighted the importance of dominant HDL diameter and HDL particle distribution profile determination for the improvement of CV risk assessment. It is generally accepted that reduced mean HDL size is associated with an increased risk of CVD development. Namely, it has been shown that elevated cholesterol ester transfer protein (CETP) activity in hypertriglyceridemia induces triglycerides (TG) enrichment of HDL particles, which upon subsequent hydrolysis become smaller and less effective in CV protection [11, 12]. In line with previous research, a higher proportion of small HDL_3 particles in pathological conditions associated with high CV risk is a consequence of structural and functional modifications of HDL_2 particles rather than *de novo* synthesis of small, protein-rich HDL_3 particles [11].

The antiatherogenic role of HDL particles is well known, and it is based on several protective mechanisms. The main atheroprotective mechanism of HDL particles is probably reverse cholesterol transport via apolipoprotein A1 (apoA1). However, HDL particles also have antioxidative effects [by the action of HDL-associated antioxidative enzymes paraoxonase 1 (PON1) and platelet-activating factor acetylhydrolase (PAFAH)], and anti-inflammatory and antithrombotic effects [9]. It is known that HDL_3 subclasses are smaller than HDL_2, and in healthy individuals, HDL_3 particles are more protective, but in conditions of hypertriglyceridemia, large particles become smaller and denser, losing their atheroprotective properties. Recently, Sulaiman et al. [13] hypothesized that the forming of fetoplacental circulation toward the end of the 1st trimester and early 2nd trimester could be associated with the synthesis of new functionally improved HDL particles that protect maternal endothelial function in an uncomplicated pregnancy. These authors also speculate that this adaptive and protective HDL-related mechanism is defective in PE, leading to the development of endothelial dysfunction.

The aim of this study was to investigate the changes in HDL particle distribution and HDL particle antioxidative capacity in pregnant women who remained at risk of PE until delivery and in women who developed PE.

Methods

Study population

For this research, we recruited 91 pregnant women whose median age was 32 years (20–46) and who qualified for this study based on being diagnosed with a high risk of PE development, and who had their regular gynecological check-ups at the Gynecology and Obstetrics Clinic "Narodni Front" in Belgrade, Serbia. The study protocol was in accordance with all relevant national regulations, institutional policies, and ethical guidelines defined by the Declaration of Helsinki. This study was approved by the Ethics Committee of the Obstetrics and Gynecology Clinic "Narodni Front" (approval number: 24/55-6, from June 14, 2016). Informed

consent was obtained from all individual participants included in the study.

According to the recommendations given by the American College of Obstetricians and Gynecologists and the National Institute for Health and Care Excellence, pregnant women were classified as having a high-risk pregnancy (HRP) if one high or two moderate risk factors for PE development were present [14, 15]. Chronic hypertension, chronic kidney disease, hypertension in previous pregnancy, high uterine artery pulsatility index, DM (type 1 or type 2), autoimmune disease, antiphospholipid syndrome, and history of thrombophilia were defined as high-risk factors, while the maternal age of 40 or older, pregnancy interval > 10 years, body mass index (BMI) > 30 kg/m^2 before pregnancy, and family history were defined as moderate risk factors [14, 15]. By the end of pregnancy, the women were divided into two groups for research purposes: 71 women, although being in the HRP group, did not develop PE before delivery and were designated as a group without preeclampsia (WPE group), while 20 HRP women (22%) who did develop PE until the end of pregnancy, were designated as a PE group. Of the 20 pregnant women who developed PE, 4 of them also had pregnancies associated with intrauterine growth restriction (IUGR), and 6 had gestational DM (GDM). PE was defined with *de novo* hypertension (≥ 140/90 mmHg) after 20 weeks of gestation, with or without proteinuria (≥ 300 mg/24 hrs), but with clinical signs of edema or organ damage. Only one woman was diagnosed with early-onset PE with delivery before 34 weeks of gestation, while 19 were diagnosed with late-onset PE. Among those who did not develop PE, 13 participants developed gestational hypertension (1 had IUGR, 3 had GDM), 2 developed IUGR and GDM, while 5 developed only IUGR and 4 only GDM until the end of pregnancy. Forty-seven (52%) participants who started their pregnancy with high or moderate risk factors for PE development carried out their pregnancies to term with no manifested complications.

Study procedures

All participants were advised to take standard vitamin and antioxidative supplements. In order to preserve their unique lipid profile, no participants were treated with lipid-lowering therapy. An exclusion criterion for participation in the study was multiple gestations.

The study was designed as a longitudinal prospective study. Blood samples were drawn after night-time fasting, towards the end of each trimester, and before the delivery [1st trimester (11–13 weeks of gestation), 2nd trimester (20–23 weeks of gestation), 3rd trimester (28–32 weeks of gestation) and before the delivery (37–38 weeks of gestation)]. Samples were collected into one serum sample tube and one EDTA sample tube, which were centrifuged at 1500xg for 10 min at 4°C. Plasma and serum samples were aliquoted and stored at -80°C until analysis.

Height and weight measurements were done for BMI calculation [BMI = weight (kg)/squared height (m^2)] and weight gain (%) assessment. Pregnancy weight gain was calculated as the difference between the last recorded maternal weight at the time when blood was sampled and self-reported maternal weight before conception. Lipid profile parameters [TG, total cholesterol (TC), HDL-C, and apoA1] were measured on Beckman auto-analyzer AU 480 employing commercial kits (Beckman, USA) in serum samples. Low-density lipoprotein cholesterol (LDL-C) concentration was calculated by the Friedewald equation [16]. The atherogenic index of plasma (AIP) was calculated according to the following equation: AIP = log (TG/HDL-C) [17]. Dominant LDL and HDL diameter, as well as HDL particle distribution, were determined by the vertical 3–31% polyacrylamide gradient gel electrophoresis method, standardized in our laboratory [18]. After electrophoretic separation, lipoprotein subclasses were assessed using Image Scanner (Amersham Pharmacia Biotech, Vienna, Austria) with Image Quant software (version 5.2; 1999; Molecular Dynamics). We determined LDL and HDL particle sizes by estimating the diameters of the most dominant peaks in the corresponding LDL and HDL regions of each scan. The relative content of each HDL subclass was approximated by determining the areas under the peaks of densitometric scans according to previously defined regions: HDL_{2b} (9.70–12.00 nm), HDL_{2a} (8.80–9.69 nm), HDL_{3a} (8.20–8.79 nm), HDL_{3b} (7.80–8.19 nm), and HDL_{3c} (7.20–7.79 nm) [19]. PON1 activity was determined by the method of kinetic spectrophotometry in serum, previously described by Richter and Furlong [20]. The concentration of paraoxon was 1.2 mmol/L, and it was purchased from Chem Service (West Chester, PA, USA). The method was modified and optimized in our laboratory [21].

Statistical analysis

The data are shown as median and interquartile range. Longitudinal changes of variables through pregnancy were measured by the Friedman test with post hoc Wilcoxon's test using Bonferroni correction for the number of mutual comparisons. Differences between two continuous variables were analyzed by the Mann-Whitney *U* test. Statistical analyses were performed using the Medcalc software (MedCalc Software, Ostend, Belgium) and PASW Statistics 18 (IBM, Armonk, New York, USA). All statistical tests were considered significant at the $p = 0.05$ probability level.

Results

The general characteristics of the study groups – the WPE group (71 participants) and the PE group (20 participants) – are presented in Table 1. Samples were taken toward the end of each trimester with numbers expressed as median (min-max): 1st trimester [12.7 (12.1–13.3) weeks of gestation], 2nd trimester [23.4 (22.7–23.8) weeks of gestation], 3rd trimester [29.6 (28.4–30.7) weeks of gestation], and before the delivery [36.7 (36.3–37.6) weeks of gestation]. In both groups, BMI significantly increased during pregnancy. As expected, systolic and diastolic blood pressure were significantly higher in the PE compared with the WPE group in almost all stages of pregnancy. We have further focused our research on lipid profile parameters in those two groups. In

the WPE group, TG, TC, and LDL-C concentrations significantly increased during the pregnancy (all $p < 0.001$), HDL-C concentration in this study group significantly increased in the 2nd trimester ($p < 0.001$) and remained increased by the end of pregnancy ($p < 0.001$), as well as apoA1 ($p < 0.001$) (Table 2). There was a significant increase in AIP values in all stages of pregnancy ($p < 0.001$), while the dominant LDL diameter significantly decreased in the 2nd trimester, and these changes persisted until the end of pregnancy in the WPE group. In the PE group, TG, TC, and LDL-C concentrations also significantly increased alongside the course of pregnancy. TG concentrations were significantly higher in the PE compared with the WPE group in all the stages of pregnancy ($p < 0.05$). We did not notice significant changes in HDL-C concentration during pregnancy in the PE group, while apoA1 significantly increased in the 2nd trimester and remained increased in all following stages of pregnancy. In the PE group, AIP significantly increased in the 2nd trimester compared with the 1st trimester ($p < 0.05$) and stayed increased by the end of pregnancy. AIP was significantly higher in the PE group compared with HRP from 2nd trimester until the delivery (Table 2).

Table 3 shows changes in HDL particle sizes and subclasses, as well as PON1 activity through pregnancy. In the WPE group, the proportions of HDL_{2b} particles significantly increased in the 2nd trimester ($p < 0.001$) and remained increased until the end of pregnancy, while proportions of HDL_{2a} ($p < 0.001$) and HDL_{3a} ($p < 0.001$) particles

Table 1

Clinical parameters in high-risk pregnancy

Group	1st trimester	2nd trimester	3rd trimester	37th WG	*p*-value
WPE					
BMI (kg/m^2)	23.6 (21.0–27.4)	25.2 ^{a**}(23.5–29.9)	26.7 ^{a**,b**}(24.6–31.1)	28.1 ^{a**,b**,c**}(25.5–32.2)	< **0.001**
Weight gain (%)	4.0 (2.0–6.0)	7.5^{a**}(5.0–10.0)	5.0^{b**}–(3.0–6.0)	5.0^{b**}(3.0–6.0)	< **0.001**
SBP (mmHg)	114.2 (102.0–123.0)	109.2 (101.1–117.6)	108.0 (102.6–121.5)	114.2^{b*}(104.0–122.6)	**0.003**
DBP (mmHg)	72.0 (63.0–80.2)	69.2 (62.5–76.0)^{a*}	71.0 (65.0–76.0)	75.5 ^{b**,c**}(69.5–81.0)	< **0.001**
PE					
BMI (kg/m^2)	27.5$^{d\#}$(24.2–31.1)	28.4$^{a**,d\#}$ (26.4–32.7)	30.0$^{a**,b**,d\#}$ (27.1–34.4)	31.2$^{a**,b**,c**,d\#}$(28.6–36.1)	< **0.001**
Weight gain (%)	4.0 (2.3–5.9)	5.5 (4.0–8.7)	5.0 (2.0–6.7)	4.6(1.2–7.0)	0.109
SBP (mmHg)	120.7 (110.6–130.2)	120.0 (105.6–126.2)	122.0$^{d\#}$ (112.6–136.5)	127.5$^{d\#}$(117.5–139.1)	**0.011**
DBP (mmHg)	77.7$^{d\#}$ (73.0–83.4)	75.2$^{d\#}$ (71.5–85.0)	80.0$^{d\#}$ (72.6–88.6)	84.0$^{b*,d\#}$(78.9–90.1)	0.166

WPE – group without the development of preeclampsia; PE – group with development of preeclampsia; BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; WG – week of gestation.
Data are expressed as median and interquartile range. Bolded values are statistically significant.
Mean significantly different from: [a] the 1st trimester; [b] the 2nd trimester; [c] the 3rd trimester; [d] the same trimester of WPE.
***p* < 0.05; ****p* < 0.001 (pairwise comparison: Friedman test with Wilcoxon *post hoc* test; Bonferroni corrected).**
#*p* < 0.05; ##*p* < 0.001 (Mann-Whitney *U* test).

Table 2

Lipid profile parameters in high-risk pregnancy

Group	1st trimester	2nd trimester	3rd trimester	37th WG	*p*-value
WPE					
TG (mmol/L)	1.29 (1.06–1.55)	1.88 ^{a**} (1.54–2.27)	2.31 ^{a**,b**} (1.80–2.95)	2.97 ^{a**,b**,c**} (2.39–3.65)	< **0.001**
TC (mmol/L)	5.2 (4.7–6.2)	6.9 ^{a**} (5.8–7.6)	7.3 ^{a**,b**} (6.0–8.6)	7.3 ^{a**,b**} (6.2–8.9)	< **0.001**
LDL-C (mmol/L)	2.9 (2.3–3.5)	3.9 ^{a**} (3.0–4.7)	4.1 ^{a**,b**} (3.2–5.2)	3.9 ^{a**} (3.2–5.1)	< **0.001**
HDL-C (mmol/L)	1.78 (1.53–2.02)	2.11^{a**} (1.85–2.45)	2.00^{a**,b*} (1.74–2.35)	1.90^{a**,b*} (1.70–2.27)	< **0.001**
ApoA1 (g/L)	1.94 (1.67–2.20)	2.31^{a**} (2.06–2.58)	2.31^{a**} (1.98–2.54)	2.25^{a**} (1.95–2.47)	< **0.001**
AIP	-0.15 (-0.24– -0.048)	-0.068 ** (-0.18–0.045)	0.045^{a**,b**} (-0.051–0.19)	0.16^{a**,b**,c**} (0.068–0.27)	< **0.001**
Dominant LDL diameter (nm)	26.3 (25.5–27.1)	25.8^{a**} (24.9–26.6)	25.2^{a**,b**} (24.7–26.0)	25.2^{a**,b**} (24.3–26.0)	< **0.001**
PE					
TG (mmol/L)	1.60$^{d\#}$ (1.19–1.97)	2.38$^{a**,d\#}$(1.75–2.92)	2.75$^{a**,b*,d\#}$(2.21–3.66)	3.62$^{a**,b**,c*,d\#}$(2.78–4.61)	< **0.001**
TC (mmol/L)	5.3 (5.1–5.8)	6.5^{a**} (5.5–7.4)	6.7^{a**} (6.0–8.1)	7.4^{a**,b*} (5.9–8.2)	< **0.001**
LDL-C (mmol/L)	2.7 (2.3–3.3)	3.2^{a**} (2.9–4.4)	3.1^{a*} (2.6–4.9)	3.1 (2.3–4.8)	**0.012**
HDL-C (mmol/L)	1.75 (1.45–1.97)	1.83$^{d\#}$ (1.66–2.10)	1.85 (1.70–2.12)	1.84 (1.73–2.16)	0.108
ApoA1 (g/L)	1.84 (1.65–2.39)	2.27^{a*} (1.95–2.68)	2.26^{a*} (2.01–2.53)	2.30^{a*} (2.02–2.67)	< **0.001**
AIP	-0.033 (-0.17–0.097)	0.13 (-0.035–0.19)$^{a*,d\#}$	0.18$^{a**,d\#}$ (0.085–0.35)	0.32$^{a**,b*,d\#}$(0.12–0.38)	< **0.001**
Dominant LDL diameter (nm)	26.7 (25.5–27.3)	25.7^{a*} (24.5–26.6)	24.8^{a**} (23.8–26.1)	24.8^{a**} (24.4–26.3)	< **0.001**

WPE – group without the development of preeclampsia; PE – group with development of preeclampsia; WG – week of gestation; TG – triglycerides; TC – total cholesterol; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; ApoA1 –apolipoprotein A1; AIP – atherogenic index of plasma.
Data are expressed as median and interquartile range. Bolded values are statistically significant.
Mean significantly different from: [a] the 1st trimester; [b] the 2nd trimester; [c] the 3rd trimester; [d] the same trimester of WPE.
***p* < 0.05; **p* < 0.001 (pairwise comparison: Friedman test with Wilcoxon *post hoc* test; Bonferroni corrected).**
#*p* < 0.05; ##*p* < 0.001 (Mann-Whitney *U* test).

Table 3

High-density lipoprotein particle size, subclass distribution, and paraoxonase 1 activity through high-risk pregnancy

Group	1st trimester	2nd trimester	3rd trimester	37th WG	*p*-value
WPE					
Dominant HDL diameter (nm)	10.81 (10.38–11.11)	10.89 (10.46–11.07)	10.75 (10.41–11.12)	10.80 (10.47–11.11)	0.594
HDL_{2b} (%)	50.91 (44.77–56.18)	54.18 (50.05–59.06)^{a**}	53.09^{a*} (47.93–59.28)	52.15 ^{a*} (47.34–58.16)	< **0.001**
HDL_{2a} (%)	20.10 (18.65–21.76)	18.98^{a**} (17.09–20.62)	18.67^{a**} (17.20–20.96)	19.57^{a*} (17.70–20.61)	< **0.001**
HDL_{3a} (%)	13.67 (12.05–15.17)	12.38^{a**} (11.05–14.28)	12.44^{a**} (10.61–13.78)	12.36^{a**} (11.04–13.97)	< **0.001**
HDL_{3b} (%)	7.90 (6.73–9.53)	7.76 (6.45–9.31)	8.09 (6.74–9.35)	8.37 (6.46–9.77)	0.389
HDL_{3c} (%)	5.58 (4.30–9.01)	5.94 (4.81–8.24)	6.79 (4.65–10.23)	6.78 (4.61–9.67)	0.249
PON1 (U/L)	284.5 (190.0–603.2)	346.5^{a**} (254.5–785.2)	358.5^{a**} (262.5–772.0)	325.0^{b*} (219.5–681.2)	< **0.001**
PE					
Dominant HDL diameter (nm)	10.88 (10.30–11.08)	10.62 (10.50–10.88)	10.67 (10.59–10.96)	10.83 (10.34–11.12)	0.569
HDL_{2b} (%)	52.16 (44.38–56.32)	57.50 (46.99–60.88)	55.06^{a**,b*} (50.63–62.43)	57.40 (45.57–61.60)	**0.001**
HDL_{2a} (%)	19.78 (16.84–22.12)	19.17^{a*} (17.47–20.18)	17.86^{a**} (15.46–18.73)	16.86$^{d\#}$ (15.88–18.96)	**0.009**
HDL_{3a} (%)	13.33 (12.26–15.67)	12.02^{a*} (11.25–14.60)	12.19 (10.98–13.79)	11.88 (10.92–14.17)	**0.001**
HDL_{3b} (%)	7.83 (6.16–9.90)	7.47 (5.82–10.06)	7.55 (6.39–9.69)	7.53 (6.22–10.06)	0.363
HDL_{3c} (%)	6.15 (5.69–8.67)	6.53 (4.31–8.06)	6.18 (4.20–7.59)	5.87 (4.70–9.30)	0.643
PON1 (U/L)	698.0$^{d\#}$ (395.5–968.0)	697.5 (379.0–1024.7)	803.5$^{d\#}$ (431.2–1305.0)	771.0$^{d\#}$ (357.7–1162.0)	0.109

WPE – group without the development of preeclampsia; PE – group with development of preeclampsia; WG – week of gestation; PON1 – paraoxonase 1; HDL – high-density lipoprotein.
Data are expressed as median and interquartile range. Bolded values are statistically significant.
Mean significantly different from: [a] the 1st trimester; [b] the 2nd trimester; [c] the 3rd trimester; [d] the same trimester of WPE.
$^{*}p < 0.05$; $^{**}p < 0.001$ (pairwise comparison: Friedman test with Wilcoxon *post hoc* test; Bonferroni corrected).
$^{\#}p < 0.05$; $^{\#\#}p < 0.001$ (Mann-Whitney *U* test).

significantly decreased in the 2nd trimester compared to the 1st trimester. In the WPE group, PON1 activity was significantly higher in the 2nd and 3rd trimesters compared with the 1st trimester. On the other hand, in the PE group, the proportions of HDL_{2a} ($p = 0.009$) and HDL_{3a} ($p = 0.001$) particles significantly decreased in the 2nd trimester. Moreover, there was a significantly lower proportion of HDL_{2a} particles in the PE group compared with the WPE group ($p < 0.05$). The PON1 activity in the PE group did not change during the pregnancy ($p = 0.109$) but was significantly higher in the PE group compared with the WPE group in the 1st and 3rd trimesters (Table 3).

Discussion

This study followed changes in the distribution and function of HDL particles in pregnant women at risk of developing PE. The lipid profile changes in uncomplicated pregnancy are similar to the ones in atherogenic pregnancy, but with appropriate compensatory and adaptive mechanisms, HDL particles manage to regulate the changes in order to avoid the development of complications in pregnancy. By monitoring pregnant women at risk of developing PE, we concluded that these adaptive changes in HDL particle function do not occur, i.e., endothelial protection is insufficient and, consequently, it is associated with clinical manifestations of PE.

A healthy pregnancy is associated with complex and intensive changes in lipid metabolism, which increase plasma lipid concentrations. These metabolic changes are necessary for the physiological course of pregnancy and fetal development [22]. In the early anabolic phase of pregnancy, increased lipid synthesis results in enlarged fat storage, while in the catabolic phase during the 3rd trimester, fat deposits have been degraded as a consequence of enhanced adipose tissue lipolytic activity [23]. These intensive metabolic changes arise as a result of a sudden and dramatic increase of estrogen, progesterone, and other reproductive hormones, as well as due to insulin resistance development, which is a physiological adaptation of pregnant women aimed to ensure adequate nutrient supply for the fetus development [23]. The main characteristics of an altered lipid profile during uncomplicated pregnancy are hypertriglyceridemia and an increase in TC and LDL-C concentrations, alongside an increase in HDL-C concentration and apoA1 [4]. In the current study, we showed that WPE is followed by changes in the size of LDL particles. The results of previous studies indicated that as a consequence of the TG concentration increase during pregnancy, the activity of CETP also increases and leads to the formation of sdLDL particles [24]. Furthermore, recently published studies have suggested that maternal predisposition for PE could be partially explained by altered lipid profile in the early course of pregnancy [5]. The results of our study showed that only TG concentrations were significantly higher in the PE group compared with the WPE during the whole course of pregnancy. That was partially the expected result because, even if it is generally accepted that PE is associated with more prominent alterations toward proatherogenic lipid profile in comparison with an uncomplicated pregnancy, in our study, all participants experienced HRP and the differences in lipid parameters between the WPE and PE groups were not so obvious. In addition, we did not notice significant changes in HDL-C concentrations through pregnancy in the PE group. HDL-C concentration is found to increase toward

the end of the 1st and the beginning of the 2nd trimester of uncomplicated pregnancy [4, 13]. We also found this pattern in the WPE group, and this increase indicates the potential protective role of HDL particles. Lipid alterations found in the PE group, an increase in TG concentration, and a lack of significant increase in HDL-C concentration could be the factors responsible for endothelial dysfunction, which is the base of PE development. Moreover, these findings highlighted the importance of AIP determination during pregnancy. Our previous results indicated that AIP, a relatively inexpensive test to perform, could be used routinely as a possible marker of pregnancy complications, especially PE [25].

This study did not notice a significant change in the dominant HDL diameter in the WPE group. We found that during HRPs, the relative proportion of large HDL_{2b} particles significantly increases in the 2nd trimester and stays increased until the delivery. This increase in large HDL_{2b} particles is associated with an increase in TC concentration and an increase in PON1 activity in the 2nd and 3rd trimesters. As we have pointed out before, the role of distinct HDL particles through HRP has been poorly investigated, and the data are inconsistent [13]. The results of our previous study indicated that during normal pregnancy, proportions of smaller, denser HDL_{3b} and HDL_{3c} particles significantly increase, and this is followed by a decrease in the proportion of HDL_{2a} subclasses [26]. Similarly, Alvarez et al. [27] reported a decrease in HDL_{2a} and an increase in HDL_{3c} subclasses in the 3rd trimester, while Silliman et al. [28] found that the final phase of a normal pregnancy is associated with an increase in large HDL_2 particles. The remodeling of HDL particles during pregnancy was explained as a subclass shift toward smaller, denser, TG-rich, and less potent particles in terms of atheroprotection [26, 27]. Recently, Sulaiman et al. [13] presented an intriguing hypothesis according to which enhanced synthesis of new HDL particles occurs as a compensatory mechanism aimed to protect vascular endothelial function in normal pregnancy. In light of this observation, we could speculate that the observed increase in small HDL_3 particles in studies of uncomplicated pregnancy could be explained as the triggering of new, protein-rich, atheroprotective, and antioxidative potent HDL particle synthesis by the placental circulation. In HRPs, this adaptive mechanism was not observed, and this could also explain the fact that almost half of these pregnancies are characterized by the development of complications.

Results of our study also confirmed an increase in PON1 activity in WPE in the 2nd and 3rd trimesters. PON1 is an HDL-associated esterase that metabolizes oxidized lipids within LDL particles, and lower PON1 activities are associated with a higher risk of CVD development [29]. Additionally, PON-1 determines the capacity of HDL to stimulate nitric oxide production and protect the endothelial function [30]. Results regarding PON1 activities in pregnancy are controversial, but it is generally accepted that PON1 activity increases through pregnancy as an additional adaptive mechanism of HDL particles in the condition of increased oxidative species formation characteristic of pregnancy [31–33].

Another important finding of our study is that alterations in HDL particles in women with overt PE are different than in those with a high risk of PE development. We did not find significant changes in the dominant HDL diameter in this group. There were no changes in HDL-C concentrations, and this result is in agreement with previous studies [4, 25]. A deeper analysis of the distribution of HDL particles through pregnancy complicated with PE showed a decrease in the relative portion of small HDL_{3a} particles in the 2nd trimester. This result brings us back to Sulaiman et al. [13] hypotheses that uncomplicated pregnancy is associated with *de novo* synthesis of HDL particles (increase in the relative proportion of small HDL_3 particles), and in PE, this adaptive mechanism is lost, which might be further associated with endothelial dysfunction development. As already mentioned, PON1 activity in the WPE group increased as a result of the adaptive mechanism, while in the PE group, there were no changes in PON1 enzyme activity. However, PON1 activity is higher in the PE group in the 1st and 3rd trimesters and before delivery compared to the WPE group, which indicates that pregnant women in the PE group needed greater antioxidant protection. PON1 was initially higher in the PE group, but this increase was insufficient to protect the pregnant women from complications [34].

Our study is limited due to the small sample size and should be confirmed in larger studies. Another limitation is the absence of a control group, which could affect the sensitivity and specificity of biomarkers as assessed in a previously selected high-risk population.

Conclusion

Overall, our results indicate that HDL particles go through structural and functional changes during HRP. The structural and functional modifications of HDL particles that go on through HRPs without the development of PE are different from those that take place in HRP with the development of PE. Dyslipidaemia in pregnancy is characterized by hypertriglyceridemia and an increase in HDL-C concentration, and this specific lipid profile constellation could be associated with adverse modifications of HDL particles, which are comparable to atherogenic dyslipidemia Adaptive pregnancy mechanisms expressed as a functional modification of HDL particles seem to lack in PE. A better understanding of the HDL particle's role in pregnancy is necessary for further investigation of pregnancy complications treatment, especially for preventing PE development.

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Conflict of interest

The authors declare no conflict of interest.

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The occurrence of depressive symptoms in rheumatoid arthritis: a cross-sectional study

Pojava simptoma depresivnosti kod bolesnika sa reumatoidnim artritisom: studija preseka

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Abstract

Background/Aim. Depression, as a common comorbidity in patients with rheumatoid arthritis (RA), has been found to affect the disease diagnosis and treatment response. Accordingly, the aim of the study was to investigate the occurrence of depressive symptoms among patients with RA and the association between RA and depression. **Methods.** The cross-sectional study included 69 patients with RA. The depressive symptoms in patients with RA were assessed using the Beck Depression Inventory (BDI), and RA activity was measured by Disease Activity Score-28 for RA with erythrocyte sedimentation rate (DAS28-ESR) and Clinical Disease Activity Index (CDAI). **Results.** It was found that 42% of respondents reported having depressive symptoms of different severity, among which the majority (23.2%) presented with mild symptoms. A mild positive correlation was found between the BDI and the DAS28-ESR (r = 0.39 p = 0.001), as well as between the BDI and the CDAI (r = 0.40 p = 0.001). Place of residence (t = -2.14 p = 0.03) and employment status (t = -2.81 p = 0.00) associated with depressive symptoms were also found to have statistically significant differences within the group of respondents. In addition, age had a positive correlation with the disease activity, as well as the place of residence and employment status. **Conclusion.** It has been observed that depressive symptoms in patients with RA were associated with disease activity, employment status, and place of residence. Therefore, there is an important need for integrating rheumatologic and mental health services for future research toward a better understanding of both depression and RA.

Key words:
age factors; arthritis, rheumatoid; depression; residence characteristics; severity of illness index; work.

Apstrakt

Uvod/Cilj. Depresija, kao čest komorbiditet kod bolesnika sa reumatoidnim artritisom (RA), može uticati na dijagnozu bolesti i odgovor na primenjeno lečenje. Zbog toga je cilj rada bio da se ispita učestalost simptoma depresivnosti kod bolesnika sa RA i međusobna povezanost između RA i depresije. **Metode.** Studija preseka obuhvatila je 69 bolesnika sa RA. Simptomi depresivnosti kod bolesnika sa RA procenjivani su korišćenjem Beck-ove skale za depresiju (*Beck Depression Inventory* –BDI), a aktivnost RA procenjivana je Indeksom aktivnosti bolesti (*Disease Activity Score-28 for RA with erythrocyte sedimentation rate* - DAS28-ESR) i Kliničkim indeksom aktivnosti bolesti (*Clinical Disease Activity Index* – CDAI). **Rezultati.** Simptomi depresivnosti različitog intenziteta zapaženi su kod 42% bolesnika, od kojih je najveći broj (23,2%) imao blage simptome depresivnosti. Utvrđena je pozitivna korelacija niskog stepena između BDI i DAS28-ESR (r = 0,39 p = 0,001), kao i između BDI i CDAI (r = 0,40 p = 0,001). Takođe, pokazano je da su mesto stanovanja (t = -2,14 p = 0,03) i status zaposlenja (t = -2,81 p = 0,00), koji su povezani sa simptomima depresivnosti, bili statistički značajno različiti unutar grupa ispitanika. Osim toga, životno doba bolesnika, mesto stanovanja i status zaposlenja su bili u pozitivnoj korelaciji sa stepenom aktivnosti bolesti. **Zaključak.** Simptomi depresivnosti kod bolesnika sa RA bili su povezani sa aktivnošću osnovne bolesti, statusom zaposlenja i mestom stanovanja. U cilju boljeg razumevanja depresije i RA, u budućim istraživanjima, neophodna je integracija reumatoloških službi i službi za mentalno zdravlje.

Ključne reči:
životno doba, faktor; artritis, reumatoidni; depresija; stanovanje; bolest, indeks, težine; rad.

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease primarily characterized by persistent synovitis symmetrically affecting peripheral joints [1]. It has been estimated that 1% of the world's population suffers from this disease [2, 3]. The majority of patients with RA exhibit a chronic fluctuating course of disease that, if left untreated, results in progressive joint damage, reduced functional ability, and disability.

Depressive symptoms are common in patients with RA. More recent studies on RA patients with depression reported that the occurrence varies between 13% and 42% due to differences in methods of identification, assessment scales used, and heterogeneity of disease presentation [4–6]. In addition, such substantial differences in the occurrence may result from the overlap between somatic depressive and RA symptoms, as well as the significant impact of perceived social support on depression [7]. Rheumatologists have estimated the occurrence of depression in 10.5% of patients with RA, while the occurrence of self-reported depressive symptoms is almost twice as common, i.e., 22% [8].

Studies have shown that depression is two times more common in RA patients than in the general population, whereby women are more susceptible to depression [9, 10]. The association between depression and RA may be attributed to many factors. In some cases, low socioeconomic status, gender, and age have been linked to the development of depression in RA patients. According to one of the studies, which takes into account the multidimensionality of socioeconomic factors, lower education and income level have been shown to be independently associated with mental health and arthritis [11]. Additionally, studies have shown that the occurrence of RA and depression is higher in women, while there is an assumption that aging increases the risk of depression. However, empirical studies investigating the relationship between aging and depression have not shown the consistency of these research findings [12]. In other cases, the occurrence of depressive symptoms is associated with the consequences of RA-related disease and disability. Loss of functional abilities is directly related to decreased activity in daily life and the presentation of depressive symptoms [13]. Consequently, depression is related to chronic pain intensity and poor clinical status, functional limitations, and disability [5, 14].

Furthermore, the psychological impact of chronic inflammatory disease on mental well-being is related to disease activity levels, as well as disease duration [15, 16]. Chronic pain, fatigue, difficulty in performing daily life activities, and psychological stress also contribute to disease activity and the manifestation of depression.

The presence of depression affects both the patient's quality of life and the disease course, increasing the need for medical care and treatment and contributing to the increase in unemployment and reduced work efficiency.

The importance of identifying depression and depressive symptoms in patients with RA has not only been associated with the treatment of comorbid depression but also with the prevention or elimination of the negative impact of depression on treatment response in patients with RA. Given the consequences of the presence of depressive symptoms associated with the course of the disease and treatment outcomes in patients with RA, we were interested in the levels of severity of depressive symptoms among patients included in this study. Specifically, the aim of the study was to determine the occurrence of depressive symptoms and the relationship between RA and comorbid depression.

Methods

The sample included 69 patients hospitalized or monitored on an outpatient basis at the Clinic for Nephrology and Clinical Immunology, University Clinical Center of Vojvodina, Serbia. All patients have been diagnosed earlier with RA by a rheumatologist.

A cross-sectional study was designed. It included patients older than 18 years of age treated with conventional synthetic disease-modifying drugs, followed by adding biological disease-modifying drugs, IL-6 receptor antagonists, or TNF-α inhibitors. Exclusion criteria were a diagnosis of depression and/or dementia before inclusion in the study, intellectual disability, patients with evidence of other systemic diseases (overlapping syndrome), and patients with a history of alcoholism or psychoactive substance abuse.

Prior to performing the planned research, each respondent was provided with both verbal and written explanations of how the research would be carried out, how the plan of research worked, and how these research findings would be implemented in practice. Afterward, they gave informed consent to participate in the study. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of the Faculty of Medicine University of Novi Sad (protocol code 01-39/212/1, date of approval July 16, 2020).

The general questionnaire was used to collect data on respondents' gender, age, occupation, marital status, and place of residence.

The level of disease activity was calculated using the Disease Activity Score-28 for RA with erythrocyte sedimentation sate (DAS28-ESR) [17]. DAS28-ESR is a combined index that measures disease activity in patients with RA based on 28 painful and swollen joint counts. Longitudinal studies have shown that the effect of disease activity and joint destruction on functional capacity changes throughout the disease. In early RA, functional capacity is mostly associated with disease activity, and in late disease, with joint damage [18]. The final score criteria classified individual patients as being in remission using DAS28-ESR ≤ 2.6, between 2.6 and 3.2 as low disease activity, between 3.2 and 5.1 as moderate activity, and > 5.1 as high activity [19].

The Clinical Disease Activity Index (CDAI) was used to determine the level of underlying disease activity [20]. It is a composite index for assessing RA disease activity based on the simple sum of the number of painful joints (28 joints) and the number of swollen joints (28 joints) and patients' and physicians' global assessment of the disease activity. The global assessment of the disease activity from both the pa-

tient and the physician was measured on a visual analog scale (VAS), a measurement instrument used commonly to asses a parameter that ranges across a continuum of values, such as pain. It ranges from 0 to 100 mm, where the lowest score indicates the absence of the examined value and is calculated by the measured distance in mm of the vertically drawn line on the scale (as a marker of intensity) from the left edge of the scale.

In addition, all patients were examined for the presence and severity of a broad range of depressive symptoms using the standardized Beck Depression Inventory (BDI). It is one of the most widely used instruments to assess depression. Since it was originally developed in English in 1961, the BDI has been translated into many languages [21]. The standardization of the Beck scale into the Serbian language was performed in 2011, and it has been shown that the results clearly indicate the fact that this scale is one of the golden standards for depression measurement and can be considered a valid instrument for depression evaluation in our surroundings [22]. BDI was designed to be used specifically as a screening tool for measuring depression symptoms and the degree of depression severity [23]. This self-report rating inventory comprises 21 items. Each item is evaluated on a severity scale ranging from 0–3 (0 = least, 3 = most), with a total score ranging from 0–63. Higher scores indicate greater depression severity. BDI cut-off scores are used to measure the severity of depression in the following way: scores from 0 to 9 indicate no depression; from 10 to 15, mild depression; from 16 to 19, mild to moderate depression; from 20 to 29, moderate to severe depression; from 30 to 63, severe depression. The BDI takes approximately ten min to complete.

As such, the BDI is considered one of the most relevant tools in assessing depression, showing high reliability and validity. Psychometric properties of the BDI with psychiatric and nonpsychiatric samples were reviewed from 1961 until June 1986. The BDI demonstrates high internal consistency, with alpha coefficients of 0.86 and 0.81 for psychiatric and nonpsychiatric populations, respectively, and the internal consistency of the scale ranged between 0.73 and 0.92 with a mean of 0.86 [24].

Statistical analysis

The research has been done in the form of an observational cross-sectional study. The SPSS 24.0 software package was used for data processing. The Kolmogorov-Smirnov test results showed harmonic data distributions of all variables compared to the nonharmonic distribution of disease activity by CDAI, which was assessed using nonparametric statistics. Descriptive statistics methods were used to measure central tendency (an arithmetic mean) and measures of variability [the standard deviation (SD)] in order to summarize the major numerical characteristics of observations. Additionally, the *t*-test was used to compare the test statistic. Mann-Whitney test was used in order to determine differences between two independent variables. Evaluation of statistically significant differences between frequency distributions among groups was verified by the Chi-squared (χ^2) test. In the tests used, statistically significant differences were observed outside the 95% confidence interval ($p < 0.05$). To measure reliability as a whole, Cronbach's alpha coefficient was used as a measure of internal consistency. The coefficients of at least 0.80 were considered acceptable.

Results

Out of the total number of respondents, 65 (94.2%) were females, and 4 (5.8%) were males. The median age of respondents was 52.2 years (SD = 13.3; min = 19, max = 75). Of the respondents, 43 (62.3%) lived in the urban area, and 26 (37.7%) lived in the rural area. According to the distribution of patients by marital status, the majority, 47 (68.1%) were married, and 22 (31.9%) were single. The distribution of respondents by status in employment was similar, where 38 (55.1%) were employed and 31 (44.9%) were unemployed. The average disease duration was 13.7 years (SD = 7.0; min = 1, max = 40).

The present study showed that the internal consistency estimate for the BDI was high, with Cronbach's alpha coefficients of 0.88. Table 1 shows average values and characteristics of parameter distribution, which were associated with the level of disease activity, functional status, and pa-

Table 1

Descriptive statistics of the data about depressive symptoms in patients with rheumatoid arthritis (n = 69) collected using research instruments

Parameter	Min	Max	Mean	SD	*Sk*	*Ku*
BDI	0.00	30.00	9.52	7.19	0.88	0.20
CDAI	0.00	57.50	11.29	9.57	2.05	7.34
DAS28-ESR	1.11	7.06	2.89	1.21	0.70	1.30
VASi	0.0	75.0	30.52	19.37	0.40	-0.65
VASb	0.0	83.0	36.33	21.23	0.36	0.70

BDI – Beck Depression Inventory; CDAI – Clinical Disease Activity Index; DAS28-ESR – Disease Activity Score-28 erythrocyte sedimentation rate; VASi – patient global health assessment measured on a visual analog scale by the physician; VASb – patient global health assessment measured on a visual analog scale by the patient; Min – minimum; Max – maximum; SD – standard deviation; *Sk* – Skewniss measurement of curvation; *Ku* – (Kurtosis) measurement of flatness.

tient disability index. As shown in Table 1, the characteristics of distribution for CDAI did not match a normal distribution, which is also identified through the values of skewness and kurtosis (values above 1.5). The remaining values from this group of parameters were presented with a normal distribution. Patients had lower mean values of BDI, CDAI, and DAS28-ESR scores. The patient-reported and physician-reported mean VAS measurement scores were approximately the same, with slightly higher scores obtained from patients. Using Intraclass Correlation (IC) for VAS ratings of the two subscales provided independently by the patient and the physician, it was obtained that there was a high degree of agreement between patients and physicians (IC = 0.94, $p = 0.00$).

The disease activity was assessed by DAS28-ESR and CDAI. The results indicated that the majority of respondents had a moderate level of disease activity. However, a larger number of patients who were in remission as measured by DAS28-ESR were more likely to have lower disease activity than those measured by CDAI (Table 2).

Regarding depressive symptoms measured using the BDI questionnaire, it was found that 40 (58%) respondents reported no symptoms, while 29 (42%) reported having symptoms. Among those who had symptoms of depression, mild symptoms were found in 16 (23.2%) respondents, mild to moderate in 5 (7.2%), moderate to pronounced symptoms in 7 (10.1%), and one (1.4%) had pronounced symptoms of depression. In the further analysis, the results were analyzed only according to the presence or absence of depressive symptomatology since in some subgroups, where the severity of depressive symptoms was assessed, there was a small number of self-reported depressive symptoms.

Pearson's Correlation showed a high-level statistical significance, but of a moderate range, between the BDI and the DAS28-ESR (r = 0.39 $p = 0.001$), as well as between the BDI and the CDAI (r = 0.40 $p = 0.001$). Nonparametric techniques of assessing differences between two groups, such as the Mann-Whitney U test and χ^2 test, were used in order to estimate whether there was a connection between certain groups of depression (those with and without depressive symptoms) and disease activity severity. These tests were also used to assess the presence of a statistically significant difference in average scores of disease activity measurements and frequency of depression symptoms. Table 3 shows the frequency values of Mann-Whitney tests, levels of statistical significance for each group, as well as the level of χ^2 test. It is indicated that the results of the Mann-Whitney U test exhibit differences in average ranges of patients in various depression categories (with and without depression), which are of a statistically significant difference in scores of the parameters shown. All of the obtained differences stand for the fact that persons with depressive symptoms had a higher disease activity index (DAS28-ESR; CDAI). Additionally, results obtained from the pain scales, measured by both the patient global health assessment measured on a VAS by the patient – VASb ($U = 253.5$ $p = 0.000$) and the patient global health assessment measured on a VAS by the physician VASi – ($U = 260.0$ $p = 0.000$), confirm that the presence of depression was associated with a higher level of pain.

In further analysis, Pearson's χ^2 test was used to determine whether there was a relationship between the levels of disease activity and depressive symptoms with some of the examined sociodemographic variables such as patient's age and disease duration. There was a relationship between depressive symptoms and respondents' status in employment, while the level of disease intensity was related to respondents' age as measured on both applied scales. The CDAI demonstrates the respondents' relationship with both places of residence and work activities (Table 4).

Table 2

Level of disease activity in patients with rheumatoid arthritis

Disease activity	DAS28-ESR n (%)	CDAI n (%)
Remission	26 (37.7)	13 (18.8)
Low	9 (13.0)	24 (34.8)
Moderate	32 (46.4)	27 (39.1)
High	2 (2.9)	5 (7.2)
Sum	69 (100.0)	69 (100.0)

DAS28-ESR – Disease Activity Score-28 erythrocyte sedimentation rate; CDAI – Clinical Disease Activity Index.

Table 3

Presence of depressive symptomatology in relation to the level of disease activity (DA)

The level of DA	Remission n (%)	Low DA n (%)	Moderate DA n (%)	High DA n (%)	U	p	χ^2	p
DAS28-ESR								
no depression	20 (50)	4 (10)	16 (40.0)	0 (0.0)	405.0	0.01	8.10	0.04
depression	6 (20.7)	5 (17.24)	16 (55.17)	2 (6.89)				
CDAI								
no depression	10 (25)	18 (45.0)	11 (27.5)	1 (2.5)	297.5	0.00	11.02	0.01
depression	3 (10.34)	6 (20.7)	16 (55.17)	4 (13.79)				

DAS28-ESR – Disease Activity Score-28 erythrocyte sedimentation rate; CDAI – Clinical Disease Activity Index.

Table 4

Relationship of some sociodemographic factors with disease levels and depressive symptoms in patients with rheumatoid arthritis

Parameter	DAS28-ESR	CDAI	BDI
Gender			
Pearson	0.86	4.02	1.01
Sig.	0.83	0.25	0.90
Place of residence			
Pearson	2.72	9.47	6.27
Sig.	0.43	0.02	0.17
Employment status			
Pearson	7.13	10.82	11.29
Sig.	0.06	0.01	0.02
Marital status			
Pearson	3.12	0.94	5.09
Sig.	0.37	0.81	0.27
Age			
Pearson	0.28	0.34	0.20
Sig.	0.01	0.00	0.09
Disease duration			
Pearson	-0.12	-0.13	0.19
Sig.	0.32	0.27	0.11

DAS28-ESR – Disease Activity Score-28 erythrocyte sedimentation rate; CDAI – Clinical Disease Activity Index; BDI – Beck Depression Inventory.

Table 5

Significance of differences between the examined variables

BDI	n	Mean	*t*-test	*p*	Cohen's *d*
Place of residence					
urban	43	8.12	-2.14	0.03	0.50
rural	26	11.85			
Employment status					
yes	38	7.42	-2.81	0.00	0.67
no	31	12.10			
Marital status					
married	47	9.04	-0.80	0.42	- 0.20
single	22	10.55			

BDI – Beck Depression Inventory.

The significance of differences examined by the *t*-test, as well as the size effect, is given in Table 5. Place of residence and working status associated with depressive symptoms also had statistically significant differences within the group of respondents. Thus, the mean value of the respondents residing in rural areas was higher, which indicated more depressive symptoms in relation to the respondents from urban areas. Effect size represents a medium. Respondents who were unemployed at the moment were more likely to have depressive symptoms, while the size effect was also moderate. In contrast to these variables, there is no statistically significant difference in the manifestation of depressive symptomatology between married and single subjects.

Discussion

According to the research conducted, the average age of respondents was 52.17 years, similar to the majority of studies in which respondents were 51–59 years old. As in other cohort studies, the majority of respondents were females, and the sample size of males in the present study was 5.8%. Previous research has shown that females reported more depressive symptoms and had poorer results obtained through different types of questionnaires measuring health-related quality of life in depression, etc. Nevertheless, there is no consensus on whether RA is worse in females or males, and that caution should be exercised when interpreting gender differences, especially related to low disease activity, which can be a consequence of measuring disease activity [25]. In further work, due to the unequal sample sizes, predominantly comprised of females, the obtained results were interpreted without taking into account gender differences.

In this study, slightly less than half (42%) of the patients had depressive symptoms exhibited in varying degrees, among which the majority of the patients had mild symptoms (23.2%). Prior studies have also reported such a high occurrence rate of depressive symptomatology depending on disease duration, the mean age, sleep disturbance, and fatigue [4, 9, 26, 27]. As can be seen from the obtained results, the reported BDI score positively correlated with the level of

disease activity measured by both DAS28-ESR and CDAI. The largest number of respondents with depressive symptoms had moderate disease activity, assessed by both DAS28-ESR and CDAI, thus suggesting that the level of disease activity influences the occurrence of depressive symptomatology in our sample.

In the high-disease-activity group, it is noticeable that, when assessed by CDAI, the number of respondents with depressive symptoms doubled compared to the same group assessed by DAS28-ESR. That could be a result of the incorporation of patient self-reported VAS in CDAI, as opposed to DAS28-ESR, and their perceived severity of the disease, which was reportedly on higher levels of disease activity. Although the level of agreement, as measured using the VAS, between respondents and observers is high, higher mean values are registered in the patient-based assessment of disease activity in terms of more pronounced problems and higher levels of disease activity. The results analyzed in this research show that when information regarding disease activity is obtained through objectively measured data, depression symptoms are registered up to a lower extent compared to the results that rely on a subjective assessment of disease activity by the patient. Considering that the assessment of mental disorders is not clearly defined in terms of a missing consensus on where the line between normal and pathological is drawn, subjective perception and interpretation of symptoms indicating depression in patients with RA must be considered. That is why in patients with RA, equal importance in functional assessment should be given to objective measurement techniques, as well as the subjective perception of patients' condition and everyday functioning.

The data obtained on the frequent presence of depressive symptoms, involving the self-evaluation of disease activity performed by the patients, only calls for the necessity of further implementation of mental health care protection. The complaints that foremost belong to the field of psychopathology are associated with everyday living and are a result of the disease symptoms but also of the lack of reaction and support of the society and surrounding they live in. Several studies report that there is a relationship between the levels of disease activity and depressive symptomatology when it comes to the likelihood of achieving remission. That depressive symptomatology is more common in the early stages of RA, but sociodemographic factors such as marital status, employment status, occupation, and patients' age also have a significant impact on their occurrence [28, 29]. That is why knowing certain risk factors and their contribution to the development of depressive symptoms in patients with RA is essential.

In our research, connections have not been found between the age of respondents and depressive symptomatology, nor with disease duration. In addition, the level of disease activity is correlated with the age of respondents, i.e., it increases with aging. Similar results are confirmed by other studies in which age was found to have a significant positive relationship with the DAS28-ESR [30, 31].

Place of residence was analyzed in relation to the respondents residing in rural areas compared to those residing in urban areas. The results obtained using the DAS28-ESR, but also BDI, have not shown any relationship with the place of residence. These findings are in line with those from the study by Movahedi et al. [32], in which no significant differences were observed demographically nor in the disease characteristics of patients living in rural and urban areas. The CDAI assessment has shown a statistically significant association with the place of residence. Since this instrument relies more on the subjective assessment of the respondents, this result may indicate that living in rural and urban areas can affect their attitudes, needs, and desires, as well as the greater availability of health services and counseling. The presence of depressive symptomatology in this study, measured by CDAI, is present more in respondents living in rural areas. Regardless of the level of disease activity, the rural environment is usually less favorable. Support services are less available, as well as the adaptation to the environment, which can affect the higher frequency of depressive symptoms occurrence. In contrast to these results, previous studies have found that rural patients are also more content with their fate [33].

However, even though RA is a chronic illness that has an impact on quality of life, functional abilities, work ability, and interpersonal relationships, no statistically significant correlations between disease activity levels and depression with marital status were registered in this study. Such findings are consistent with previous research suggesting that RA is unrelated to marital status [34].

The presence of symptoms of depression differs in relation to the respondents' employment status, i.e., depressive symptoms are related to a higher risk of unemployment status. Several studies have revealed that people with RA are more likely to be absent from work, need more time to complete work activities, have difficulty with physical tasks, and have fatigue development, often accompanied by a reduction in earnings and job losses. The loss of work productivity among RA patients is associated with the level of disease severity, as well as the presence of depressive symptomatology. Unemployed patients, bearing in mind that it is a chronic, progressive disease, gain an insight into their disease that will lead to the need for reduced working hours, lower productivity, and inability to engage in work-related tasks and personal activities, which affects the occurrence of depressive symptomatology.

The study did not include the assessment of the quality of life in people with RA or co-existent comorbidities, which could be considered a limitation of the study. These factors could also affect the occurrence of depressive symptomatology among these individuals to a certain degree and have mutual influence. The study sample was small and derived from a single center, which could also be considered a limitation. The impact of specific disease-modifying drugs used in RA treatment was not considered but should be considered for further research implementation. The abovementioned data would provide additional information on depressive symptoms in RA, thus providing the basis for further research.

Conclusion

Even though depressive symptoms have been identified in almost half of the patients, most patients have mild depressive symptoms. The presence of these symptoms is correlated positively with the level of disease activity, which speaks in favor of the fact that this symptomatology is an important factor that may influence further prognosis and treatment of these patients. A higher occurrence of depressive symptoms was identified in situations where patients self-evaluated their disease activity, which only shows the need to take greater care of their mental health. The analysis of certain risk factors and their influence on the appearance of depression indicated that work status and place of residence contribute to the appearance of depressive symptoms, especially when it comes to the subjective evaluation of the patients. That is exactly why adequate evaluation of risk factors and support systems is necessary for RA patients and should be based on patients' needs through coordinated services from different field experts. It is important to conduct further observational studies, which could give more information on mental health disorders associated with RA and thus help patients and physicians in decision-making and treatment.

Conflict of interest

The authors declare no conflict of interest.

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Root resorption of adjacent teeth due to maxillary impacted canines – comparative analysis of the findings on cone beam computed tomography and panoramic imaging

Resorpcija korenova susednih zuba izazvana impaktiranim maksilarnim očnjacima – uporedna analiza nalaza na snimku kompjuterizovane tomografije konusnog zraka i panoramskom snimku

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Abstract

Background/Aim. A frequently reported phenomenon associated with impacted maxillary canines is root resorption (RR) of the adjacent teeth. The reported incidence of RR also depends on the radiographic imaging method used. The aim of the study was to evaluate the correlation between two radiographic methods: panoramic imaging (orthopantomogram – OPT) and cone beam computed tomography (CBCT), in diagnosing contact between the impacted canine with the adjacent teeth and the existence of their resorption. **Methods.** The study included 64 subjects aged 12 to 33 years, with 80 impacted maxillary canines not orthodontically treated previously. Positions of impacted maxillary canines and possible RR of adjacent teeth were firstly estimated on the OPT and then on the CBCT**. Results.** The estimated prevalence of RR of permanent teeth was significantly different concerning the estimation of OPT and CBCT imaging. RR of the adjacent teeth was found in 25% of the OPT but in 66.25% of the CBCT. The lateral incisor was the tooth most commonly affected by RR. It is especially important to emphasize that premolar resorption was not detected at all using OPT. **Conclusion.** There was a highly significant difference between OPT and CBCT analysis concerning the relationship between the impacted canine and adjacent teeth and their possible resorptions. CBCT is a more accurate and precise examination method compared to OPT for determining the localization of impacted teeth and the possible presence of RR in the adjacent teeth.

Key words:
cone-beam computed tomography; cuspid; radiography, panoramic; root resorption; tooth, impacted.

Apstrakt

Uvod/Cilj. Česta pojava koja se javlja kao posledica impaktiranih maksilarnih očnjaka jeste resorpcija korenova (RK) susednih zuba. Učestalost otkrivenih RK zavisi i od korišćene radiografske metode. Cilj rada bio je da se uporedi pouzdanost dve radiografske metode: panoramskog snimka (*orthopantomogram* – OPT) i kompjuterizovane tomografije konusnog zraka (*cone beam computed tomography* – CBCT) u dijagnostici kontakta impaktiranog očnjaka i susednih zuba, kao i postojanja resorpcije njihovih korenova. **Metode.** U studiju su bila uključena 64 ispitanika, starosti od 12 do 33 godina, sa 80 impaktiranih maksilarnih očnjaka, koji prethodno nisu bili ortodontski tretirani. Položaj impaktiranih maksilarnih očnjaka i moguća RK susednih zuba ispitivani su najpre na OPT snimku, a potom na CBCT snimku. **Rezultati.** Procenjena učestalost RK susednih zuba bila je statistički značajno različita na OPT i CBCT snimku. Utvrđeno je 25% resorpcija na OPT, a 66,25% na CBCT snimcima. RK su bile najučestalije na lateralnim sekutićima. Posebno je važno istaći da ni jedna resorpcija na premolarima nije otkrivena na OPT-u. **Zaključak.** Postoji statistički značajna razlika u nalazu RK susednih zuba izazvanom impaktiranim maksilarnim očnjakom utvrđena analizom OPT i CBCT snimaka. CBCT je tačnija i preciznija metoda ispitivanja u poređenju sa OPT u određivanju položaja impaktiranog zuba i eventualnog prisustva RK susednih zuba.

Ključne reči:
kompjuterizovana tomografija konusnog zraka; očnjaci; ortopantomografija; zub, koren, resorpcija; zub, impakcija.

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Introduction

Impaction of maxillary canines is a frequently encountered clinical problem, the treatment of which usually requires an interdisciplinary approach. The maxillary canines are commonly impacted teeth, second only to third molars, with a prevalence of approximately 1–3% [1, 2]. The most frequently reported complication associated with the occurrence of impacted maxillary canines is root resorption (RR) of the adjacent teeth.

RR is an asymptomatic phenomenon defined as a progressive loss of cementum and dentine. Its diagnosis is essentially radiographic. Panoramic radiography is the most frequently used diagnostic imaging method in the treatment planning of impacted maxillary canines. However, often panoramic radiography does not provide enough information in treatment planning for safely performing orthodontic treatment of impacted canines. For RR associated with impacted teeth, cone beam computed tomography (CBCT) –scans provide substantially superior visualization of roots than routine radiographs by eliminating artifacts resulting from the superimposition of structures and depicting the 3D root structure from all possible directions [3, 4].

The reported incidence of RR also depends on the radiographic imaging method used. Conventional periapical radiography appeared as an inaccurate method for diagnosing RR.

The first study on the prevalence of incisor RR due to displaced or impacted canines was examined by the standard two-dimensional intraoral X-ray techniques in 1987. The canine impaction was found to cause RR on maxillary incisors in 12% of cases [5]. After 12 years, the same problem was examined in a study using the computed tomography scan, and the number of found cases increased to 48% [6]. When the combination of panoramic views and lateral cephalographs is used, RR may be overlooked in 50% of cases [7–9]. Today, it is quite clear that CBCT is an important stage in making diagnoses of impacted canines and treatment planning. This three-dimensional (3D) technique can provide overlap-free sagittal, axial, and coronal images for the dental structure in question. According to recent literature, by analyzing CBCT images, up to 70% of impacted maxillary canines cause RR of at least one adjacent tooth [10–12]. Previous studies have shown that diagnostic accuracy significantly increased with the use of 3D visualization than with panoramic views and cephalographs [13].

For generalized RR or that associated with impacted teeth, CBCT scans provide more sensitive and accurate information than periapical or panoramic radiographs. Thus, detection of slight to moderate pretreatment RR by CBCT, that may go undetected by panoramic imaging, could lead to modifications in borderline cases to reduce the duration of treatment and magnitude of tooth movement to mitigate additional RR; therefore, it can have an impact on treatment planning [14, 15].

As a result of impacted canines, RR seems to be a rapid, progressive process that almost always ceases once the impacted canine has been removed from the affected root area. Lateral incisors with RR may not exhibit clinical symptoms and may show good long-term healing and prognosis after canine extraction [16, 17].

The aim of the study was to correlate two radiography methods – panoramic imaging (orthopantomogram – OPT) and CBCT, in evaluating the relationship of maxillary impacted canine and adjacent teeth and diagnosing the existence of their RR.

Methods

The study included patients referred for consultation and treatment of maxillary impacted canine to the Department of Orthodontics at the Faculty of Medicine, University of Priština in Kosovska Mitrovica, between 2015 and 2019. This study included 64 subjects aged 12 to 33 years, with 80 impacted maxillary canines without previous orthodontic treatment. A standard examination by an orthodontist was performed in all subjects, and the absence of one or both maxillary permanent canines or the persistence of deciduous canines was determined. To confirm the clinical findings, subjects were referred for OPT imaging because of ectopic eruption of one or both maxillary canines. After clinical and radiographic examinations, those canines that did not erupt were defined as impacted canines in this study. We defined any case as maxillary canine impaction if the root formation was 2/3 complete or if the other side of the maxillary canine had erupted completely. Patients presenting cysts related to studied impacted canines, as well as patients with supernumerary teeth or missing lateral incisors or premolars, were excluded from further analysis. The study was carried out by analyzing a CBCT of the maxilla two months after OPT analysis, performed in order to plan upcoming orthodontic treatment. Informed written consent was obtained from all the subjects.

For every impacted canine, the following parameters were recorded: type of impaction (unilateral, bilateral); the labio-palatal position of the impacted canines (buccal, palatal, or mid alveolar); RR of the adjacent tooth. If the RR was suspected, resorption was graded for each tooth separately, based on the system suggested by Ericson and Kurol [6], into 4 categories: no resorption (intact root surface, the cementum layer may have been lost), slight resorption (resorption up to half of the dentine thickness), moderate resorption (resorption of the dentine midway to the pulp or more, the pulp lining being unbroken), and severe resorption (resorption reaches the pulp); The localization of RR (the cervical, middle, or apical third of root) was also recorded.

First, the given parameters were measured on the OPT. After panoramic radiography analysis, CBCT was performed in order to diagnose, plan, and prevent complications during future orthodontic treatment.

Descriptive statistics were used to describe the basic features of the study data and methods for evaluating the agreement between CBCT and OPT measurements. Statistical results were tested at a level of statistical significance (*alpha* level) of 0.05 (*kappa* coefficient [18]).

Results

In this study, a total of 64 participants with OPT images and CBCT scans were included, and 80 impacted canines were analyzed retrospectively. The mean age of subjects was 16.3 ± 4.3 years. Of the 64 included patients, 23 (35.9%) were male, so there was a statistically significant difference concerning patients' gender ($p < 0.001$).

Unilateral impaction was present in 48 (75%) patients, and 16 (25%) patients presented with bilateral impaction. The analyses of the three-dimensional location revealed that most of the impacted canines were located in the palatal position – 58 (72.5%), 19 (23.75%) were located in the buccal position, and only 3 canines (2.75%) were in the middle of the alveolar process.

In our study, we found 20 (25%) RRs on OPT images – 14 (17.5%) on the lateral incisors, 6 (7.5%) RRs on the central incisors, and no RRs on the first premolars. However, on CBCT scans, we detected 53 (66.25% of the affected quadrants) RRs – 29 (36.25%) on the lateral incisors, 19 (23.75%) on the central incisors, and 5 (6.25%) on the first premolars (Figure 1).

The lateral incisor was the tooth most commonly affected by RR due to the presence of an impacted canine. The reported prevalence of RR of permanent teeth showed significant differences between OPT and CBCT imaging (Tables 1 and 2).

Out of detected 36.25% of RRs on the lateral incisors, the resorption was located in the apical third of the root in 12 (15%), the middle third of the root in 14 (17.5%), and the

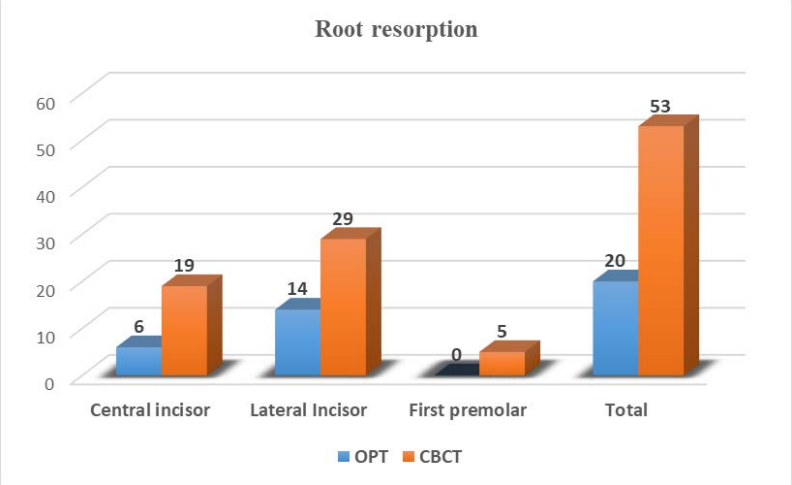

Fig. 1 – Root resorption (RR) of adjacent teeth.
OPT – orthopantomogram; CBCT – cone beam computed tomography.
k = 0.164; 95% confidence interval (CI) = 0.023–0.351 (*kappa* values: no agreement, < 0; slight, 0–0.2; fair, 0.21–0.40; moderate, 0.41–0.60; substantial, 0.61–0.80; almost perfect, 0.81–1) [18].

Table 1

Location of root resorption on lateral incisors using orthopantomogram (OPT) and cone beam computed tomography (CBCT) imaging

Parameter	CBCT				Total
	no	apical third	middle third	cervical third	
OPT					
no	46	8	9	3	66
apical third	1	3	2	0	6
middle third	3	1	3	0	7
cervical third	1	0	0	0	1
Total	51	12	14	3	80

Table 2

Severity of root resorptions on lateral incisors using orthopantomogram (OPT) and cone beam computed tomography (CBCT) imaging

Parameter	CBCT				Total
	no	slight	moderate	severe	
OPT					
no	46	9	9	2	66
slight	4	1	4	0	9
moderate	1	0	1	1	3
severe	0	1	0	1	2
Total	51	11	14	4	80

cervical third of the root in 3 (3.75 %) impacted lateral incisors; 11 (13.75%) RRs were considered slight, 14 (17.5%) moderate, and 4 (5%) severe.

This study showed differences between the two images regarding RR on lateral incisor, which was statistically significant concerning both images; a poor agreement was found between the two methods for the location of RR (κ = 0.218; 95% CI = 0.027–0.409) and its severity (κ = 0.179; 95% CI = 0.006–0.363) (Figure 2).

OPT image and CBCT very often show different findings of RRs on lateral incisors (Figure 3).

According to our results, the central maxillary incisors were affected by RR second to lateral incisors. In some cases, the impacted canine was resorbed by lateral and central incisors, together. Only one impacted canine crossed the transversal midline and it was resorbed by two central incisors. The RRs were found in 6 (7.5%) central incisors on the OPT images but in 19 (23.75%) central incisors on the CBCT scans. Results concerning RRs of central incisors are shown in Tables 3 and 4.

Most often, the resorption was located in the middle third of the root in 8 (10% of the total 23.75%) central incisors, in the apical third of the root in 5 (6.25%), and 6 (7.5%) in the cervical third of the root. Seven (8.75%) resorptions were considered slight, 9 (11.25%) moderate, and 3 (3.75%) severe.

The RR on central incisors showed a poor and very poor agreement between OPT images and CBCT scans (Figure 4).

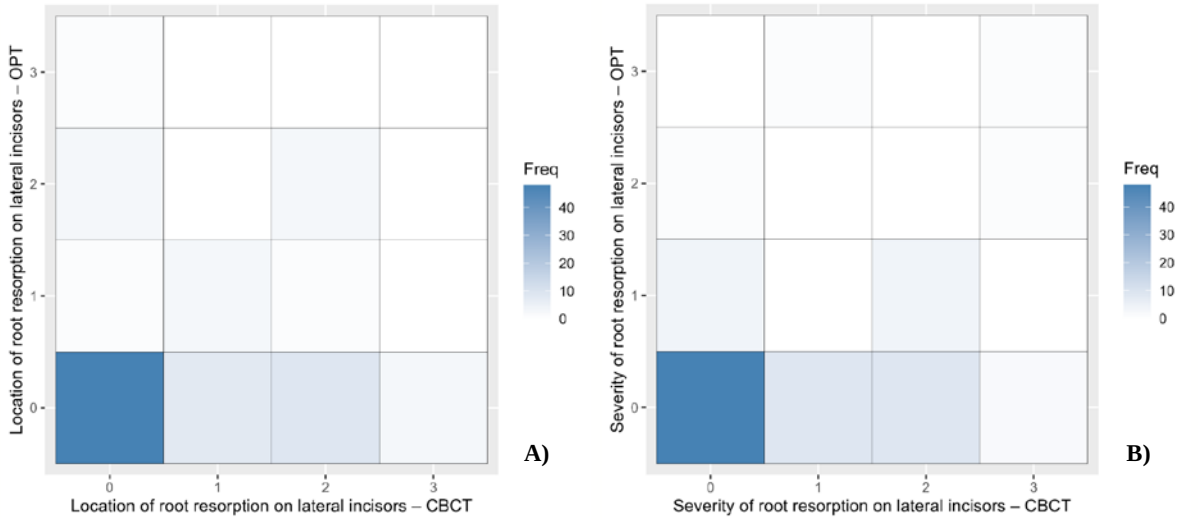

Fig. 2 – A) Agreement for the location of root resorption on lateral incisors on orthopantomogram (OPT) and cone beam computed tomography (CBCT) (κ = 0.218; 95% CI = 0.027–0.409); B) Agreement for the severity of root resorption on lateral incisors on OPT and CBCT (κ = 0.179; 95% CI = 0.006–0.363).

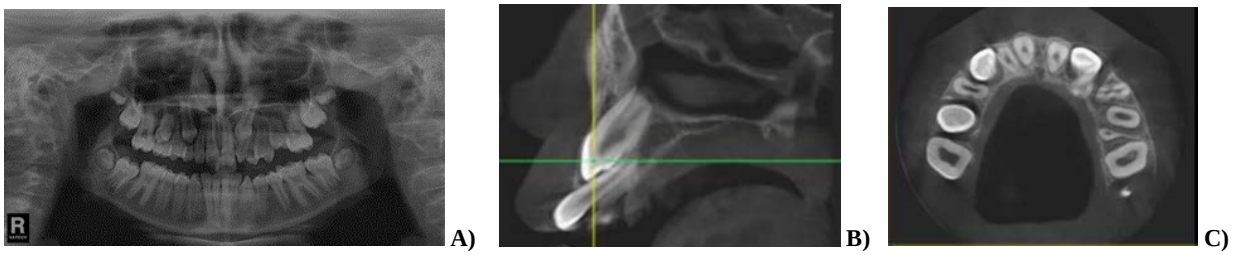

Fig. 3 – A 13-year-old patient. A) Orthopantomogram (OPT) image shows a buccal left impacted canine which overlaps with lateral incisor, with suspected moderate root resorption (RR) on lateral incisor; B) The sagittal plane on cone beam computed tomography (CBCT) scan shows RR in the apical third of the root on lateral incisor; C) Axial plane on CBCT shows slight RR.

Table 3

Location of root resorption on central incisors using orthopantomogram (OPT) and cone beam computed tomography (CBCT) imaging

Parameter	CBCT				Total
	no	apical third	middle third	cervical third	
OPT					
no	57	4	8	5	74
apical third	2	1	0	0	3
middle third	2	0	0	0	2
cervical third	0	0	0	1	1
Total	61	5	8	6	80

Slight RRs on adjacent teeth are most commonly omitted on OPT (Figure 5).

It is especially important to emphasize that premolar resorption was not detected using OPT images, but after CBCT analysis, we found 5 (6.25%) resorptions of the first premolars – 2 were moderate resorptions in the middle third of the root, and 3 were slight resorptions in the apical third of the root. These resorptions were found with palatal impacted canine with distal inclination, and in the second case, the cause is the transposition of the buccal impacted canine and the first premolar.

Discussion

The comparative analysis of our study confirmed that CBCT provides more precise information in diagnostic analysis, especially for planning orthodontic and surgical procedures where complications can be expected due to the close relationship of maxillary impacted canine and adjacent teeth.

Over the years, clinicians have searched for clues that may indicate a high risk for incisor RR associated with impacted maxillary canines. CBCT enables determining the exact distance of adjacent teeth; such a relationship is almost

Table 4

Severity of root resorption on central incisors using orthopantomogram (OPT) and cone beam computed tomography (CBCT) imaging

Parameter	CBCT				Total
	no	slight	moderate	severe	
OPT					
no	57	6	8	3	74
slight	1	1	1	0	3
moderate	2	0	0	0	2
severe	1	0	0	0	1
Total	61	7	9	3	80

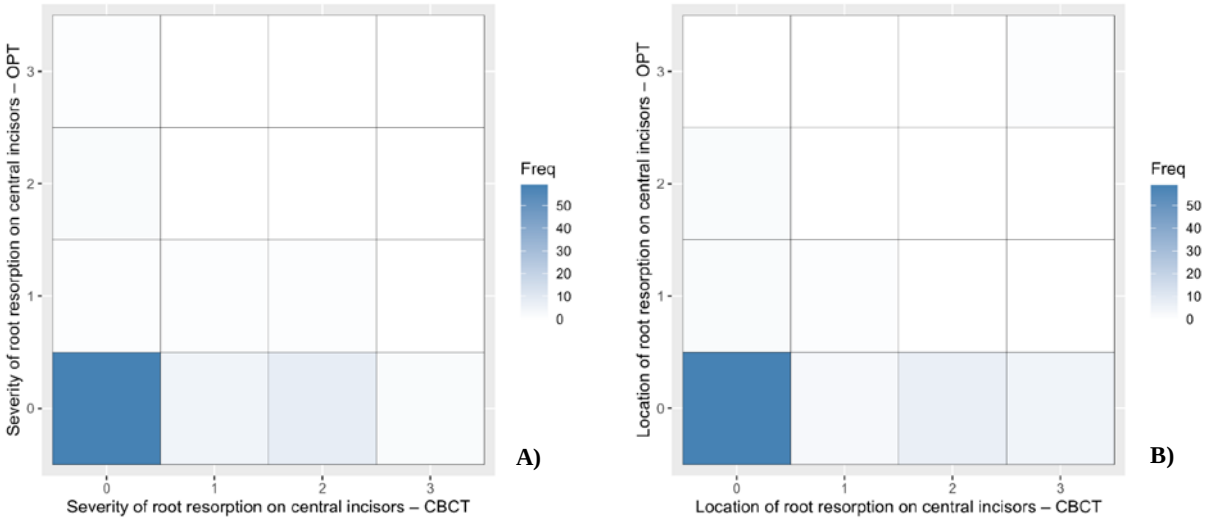

Fig. 4 – A) Agreement for the location of root resorption (RR) on central incisors on orthopantomogram (OPT) and CBCT (κ = 0.109; 95% CI = 0.102–0.320); B) Agreement for the severity of RR on central incisors on OPT and CBCT (κ = 0.016; 95% CI = 0.118–0.149).

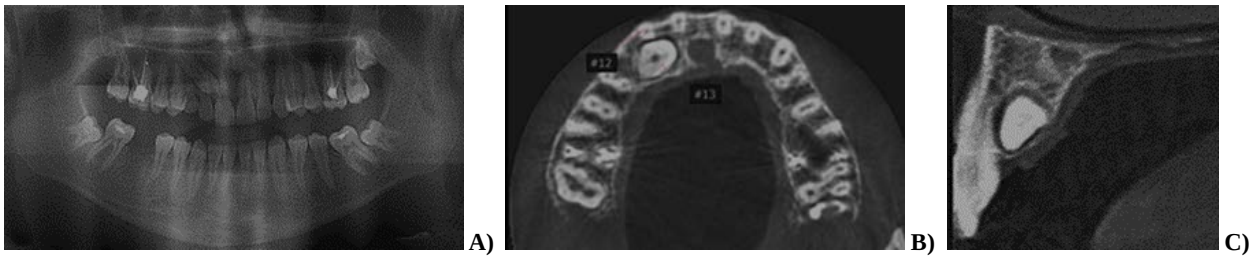

Fig. 5 – A 16-year-old patient. A) Orthopantomogram (OPT) image shows a palatally right impacted maxillary canine and no root resorption (RR) on adjacent teeth; B) Axial plane on cone beam computed tomography (CBCT) scan shows slight RR on central incisor; C) Sagittal plane shows resorption in the middle of the root.

impossible to assess accurately on OPT. Any distance of an impacted canine less than 1 mm implies contact with the adjacent tooth; in many instances, that contact also causes RR of the adjacent teeth [19, 20].

In our study, we found RRs in 25% of OPT images (17.5% on the lateral incisors, 7.5% on the central incisors, and no resorptions on the first premolars). However, on the CBCT scans, we detected much more cases (66.25%) with RR. Our findings confirmed that not every resorption of the permanent root was detected on panoramic imaging. RR may be overlooked in many cases on the OPT, such as first premolars and many resorptions on incisors. There was poor agreement between CBCT and OPT in assessing the resorption of permanent adjacent teeth.

Botticelli et al. [21] found only 5.6% of RRs on incisors using OPT, but 15.6% of RRs on CBCT. As a result of a sophisticated future and improved "cone-beam" method, images of impacted maxillary canines detect RRs even in 66.7% of lateral and 11.1% of central incisors [22]. With the same sample of the impacted canines as ours, Rafflenbeul et al. [23] actually found two-thirds of resorptions in untreated patients, while in our country, this result is higher than 65% of resorptions.

In our study, as well as in many other, maxillary lateral incisors were found to be the most affected teeth, followed by maxillary central incisors. We found similar results in other publications as well [9, 19, 24]; however, other studies showed different results concerning first premolars – the first premolars were more often resorbed than the central incisors [23, 25].

There is disagreement in the perception of the location of RR of compatibility in the results of the OPT and CBCT regarding the severity of RR and its localization. Other authors also found similar results [13, 14, 25–27]. This enhanced information, derived from the CBCT scans relative to the OPT images, may be critical in changing treatment plans. Although such treatment decisions appear to be a logical clinical outcome with the use of CBCT, the effects of the superior information derived from CBCT images may influence treatment decisions.

Of all adjacent teeth examined, resorption was most present in the middle and apical thirds of the root. Severe resorption has been presented the least, but slight and moderate resorptions have been similarly presented. Regarding the localization and severity of incisor RR, we found similar results in other publications [10, 19, 23–28].

Thereby, diagnosed resorption usually does not change prior to orthodontic treatment but significantly affects the treatment plan in terms of determining the direction of orthodontic traction. Otherwise, resorption existing on the adjacent teeth may become worse by displacing the impacted canine. This predominance is confirmed by all studies, excluding patients with past or ongoing orthodontic treatment. Early diagnosis and treatment are imperative [27, 29-31]. The prevalence of moderate and severe resorptions tends to be higher in most other studies, perhaps because in cases of past or ongoing orthodontic treatment, poor control of the relationship between the canine and the adjacent roots could have worsened already present RRs at a lesser extent.

Our further research will be focused on monitoring diagnosed resorptions during orthodontic treatment and expansion of impacted maxillary canines – whether and how they will change.

Conclusion

The diagnosis of the presence of RR was significantly different between the OPT images and the CBCT scans. Accordingly, 25% of RRs were found on OPT images but 66.25% on CBCT scans. There was a highly significant difference between OPT and CBCT in analyzing the relationship between the impacted canine and adjacent teeth and their resorption. These results showed that OPT and CBCT images of impacted maxillary canines could produce different diagnoses and treatment plans.

Conflict of interest

The authors declare no conflict of interest.

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Determination of spatial position of tibial graft using X-ray images after anterior cruciate ligament reconstruction

Određivanje prostornog položaja grafta golenjače korišćenjem rendgenskog snimka posle rekonstrukcije prednjeg ukrštenog ligamenta

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Abstract

Background/Aim. Anterior cruciate ligament reconstruction is one of the most commonly performed knee surgeries in young adults. The success of this procedure largely depends on the proper formation of the tunnel, which is obtained by drilling the tibia and which serves to position and fix the graft. The aim of the study was to present a method for determining the spatial position of the graft based on only two standard X-rays. **Methods.** The study was performed on a group of 15 patients in whom the developed software applied the measurement of the angle of the tunnel in the tibia based on the selection of characteristic points on two standard X-rays of the knee (anterior-posterior and lateral projection). The obtained results were compared with the results of measuring the angle of the tunnel in the tibia on knee images by computed tomography (CT) in all patients. **Results.** The drilling angle measured in CT scans was, on average, somewhat greater (59.07° ± 5.61°) than the angle measured by applying a developed application (58.65° ± 5.89°). The obtained results indicated minimal differences without statistical significance in the measurements of the angle of the tunnel in the tibia using the developed software and on CT images (Wilcoxon test: Z = -1.363; p = 0.173). **Conclusion.** The presented method and developed software are suitable for everyday clinical applications in terms of precision and usability and can be used to assess the position of tunnels in the tibia in the process of determining the success of surgery or in preparing patients for revision surgery.

Key words:
anterior cruciate ligament reconstruction; computer-aided design; orthopedic procedures; radiography; tibia.

Apstrakt

Uvod/Cilj. Rekonstrukcija prednjeg ukrštenog ligamenta jedan je od najčešće izvođenih hirurških zahvata kolena kod mladih osoba. Uspešnost tog zahvata u velikoj meri zavisi od pravilnog formiranja tunela koji se dobija bušenjem golenjače i koji služi za pozicioniranje i fiksiranje kalema. Cilj rada bio je da se prikaže metoda za određivanje prostornog položaja grafta na osnovu samo dva standardna rendgenska snimka. **Metode.** Istraživanje je izvršeno na grupi od 15 pacijenata kod kojih je razvijenim softverom primenjeno merenje ugla tunela u golenjači, na osnovu selekcije karakterističnih tačaka na dva standardna rendgenska snimka kolena (prednje-zadnja i bočna projekcija). Dobijeni rezultati upoređeni su sa rezultatima merenja ugla tunela u golenjači na snimcima kolena kompjuterizovanom tomografijom (KT) kod svih pacijenata. **Rezultati.** Ugao bušenja meren na KT snimcima u proseku je bio nešto veći (59,07° ± 5,61°) od ugla merenog primenom razvijene aplikacije (58,65° ± 5,89°). Dobijeni rezultati ukazuju na minimalne razlike, bez statističke značajnosti, u merenjima ugla tunela u golenjači, primenom razvijenog softvera i na KT snimcima (*Wilcoxon test*: Z = -1,363; p = 0,173). **Zaključak.** Prezentovana metoda i razvijeni softver pogodni su za svakodnevnu kliničku primenu sa stanovištva preciznosti i upotrebljivosti i mogu se primeniti za procenu položaja tunela u golenjači u procesu utvrđivanja uspešnosti operativnog zahvata ili u sklopu pripreme pacijenta za revizionu operaciju.

Ključne reči:
ligament, prednji, ukršteni, rekonstrukcija; kompjuterski podržan dizajn; ortopedske procedure; radiografija; tibija.

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Introduction

The anterior cruciate ligament (ACL) is one of the four most important ligaments responsible for knee stability in a stationary position and when in motion. It prevents anterior tibial movement in relation to the femur and plays a significant part in ensuring lateral and rotational knee stability (Figure 1) [1].

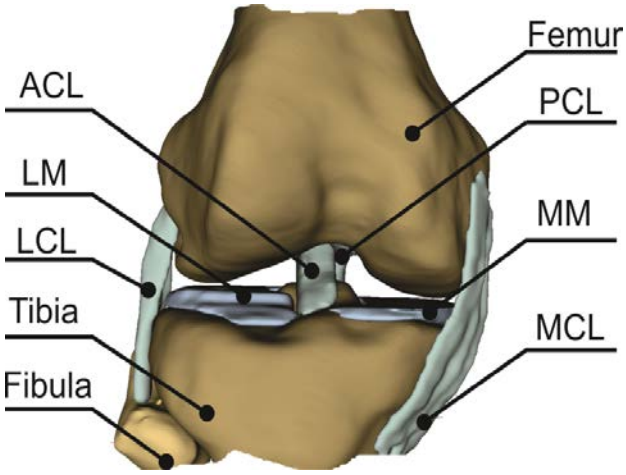


Fig. 1 – Ligamentous apparatus of the knee joint.

ACL – anterior cruciate ligament; LM – lateral meniscus; LCL – lateral collateral ligament; PCL – posterior cruciate ligament; MM – medial meniscus; MCL – medial collateral ligament.

Successful ACL reconstruction by arthroscopic surgery reduces total treatment costs, shortens the rehabilitation period, and enables the quicker return of the patients to their everyday activities [2].

In the last three decades, the middle third of the patellar ligament has been used in ACL reconstruction as a replacement for the damaged one. This technique has become a "gold standard" over time [3]. However, semitendinosus and gracilis muscle tendons have been used in the last decade, as well as allografts; bone-patellar tendon-bone allograft is the most frequently used, while Achilles tendon allograft and the iliotibial band are rarely used [4]. Allografts are being used more frequently due to the increased number of recurrent ACL reconstructions and their numerous advantages in practical use. Some of the advantages are the following: there are no local complications in the region of autograft harvest (pain, crepitation, weakness of femoral muscle, etc.); the timing of surgery is shortened; a smaller incision after embedding the autograft is required; rehabilitation is easier and faster. Due to all these reasons, the allograft is a lot more economically efficient [5]. Each of these techniques has its advantages and disadvantages and is the subject of numerous research and a topic of controversy [6–8].

Despite the development of surgical techniques and rehabilitation, revision surgery is required in some patients due to unfavorable outcomes of ACL reconstruction.

The causes that result in recurrent knee instability after ligament reconstruction are failure of surgical technique, problems in relation to the used graft, and undetected knee instability. Improper graft position is considered the most frequent cause of early recurrent instability, i.e., the position of the tunnel created by drilling with the aim of graft positioning and fixating that is not at its anatomical site [9, 10]. Since graft position depends on the femoral and tibial tunnel placement, there is a popular belief that drilling the tunnels in the position within their anatomical insertion during ACL reconstruction is necessary. In that way, anatomical ACL reconstruction could be achieved, stability and normal knee kinematics regained, and the patient would recover more quickly [11].

Most surgeons who deal with this issue determine graft position (i.e., the tunnel where the graft is situated) in the tibia by measuring according to standard X-ray images: anterior-posterior and lateral projection, which certainly is not precise enough [12, 13]. The position of the graft can also be determined by analyzing images of computed tomography (CT) or magnetic resonance (MRI) along with spatial reconstruction in the knee region or by creating a specialized application that determines spatial graft position using standard X-ray images, which is the goal of this research.

The aim of the study was to present a method for determining the spatial position of the graft based on only two standard X-rays, as opposed to CT or MRI imaging which are more expensive, and patients are exposed to lower doses of ionizing radiation compared to CT imaging. Furthermore, specialized software has been developed for everyday clinical applications, and it can be used to assess the position of tunnels in the tibia in the process of determining the success of surgery or in preparing patients for revision surgery.

Methods

Surgery

ACL reconstruction was performed in a group of patients using a bone-patellar tendon-bone graft. During the surgery, the patients were lying on the back with their legs on the arthroscopic leg holder, in general, spinal, or epidural anesthesia. After the graft was harvested, the processing began. Simultaneously, arthroscopy was performed to verify ACL tear and detect possible joint lesions (osteochondral lesion, loose joint body, meniscus injury, chondromalacia of cartilage). After that, the tunnel was drilled through the tibia and femur using a drill of 9 mm or 10 mm in diameter. When the drilling of the tunnel was finished, the graft was inserted and fixated by cannulated screws of dimensions 8 × 25 mm. Finally, graft position and its relation to the walls of the intercondylar fossa in the position of the maximum extension were checked once again by arthroscopy. The study was approved by the Ethics Committee of the Faculty of Medicine in Novi Sad, Serbia (Nº 01-39/137/1 from 03 February, 2017).

Determination of graft angle

The determination of spatial graft angle was based on X-ray images of the knee region in two orthogonal projec-

tions (in the sagittal and coronal planes). Given that the patient's knee position and orientation can never be ideal due to the measuring angles, the first phase of determining graft spatial angles involved placing orthogonal projections in the scans in the proper position. The procedure involved the determination of common points that enable precise projection definitions of the appropriate knee surfaces. Based on the clinical procedure for evaluating the tunnel angle, the procedure which defines the specific points on the tibial plateau and tunnel has been accepted (Figure 2). Figure 3 shows separate points with the planes they define and the appropriate angles they form with the graft axis.

The reference coordinate system (point P) was set at the intersection point of the tunnel where the graft was positioned and in the plane of the tibial plateau. To harmonize projections, this plane needed to be seen as a horizontal line in both projections (*x*-*y* plane). The first step in this procedure was to evaluate tibial plateau angles in both projections concerning the horizontal axis (angles γ and δ) (Figure 3).

With the rotation of plateau and tunnel projections around the coordinate system starting point (P) for the previously evaluated angles, the connected projections were obtained (Figure 4a). These modified views display the coordinates of the graft endpoint: from the front view, *x* and *z* coordinates, whereas the *y* coordinate was obtained from the lateral view. The graft intersection point with the tibial plateau (coordinate start) represents the other point that determines the tunnel axis (Figure 4b).

The tunnel drilling angle (sagittal angle – α) was calculated between the generated spatial graft line and its projection into the *x*-*y* plane. Transversal angle (β) was calculated between the graft line projection into the *x*-*y* plane and a unit vector in the direction of the *y*-axis (Figure 4b). In order to see the drilling angle in its full size from the lateral view, it was necessary for the transversal angle to be 0º, i.e., to make a scan in the *y*-*z* plane so that the rays were normal on the drilling plane. Given that graft is required to be as close as possible to the anatomical position,

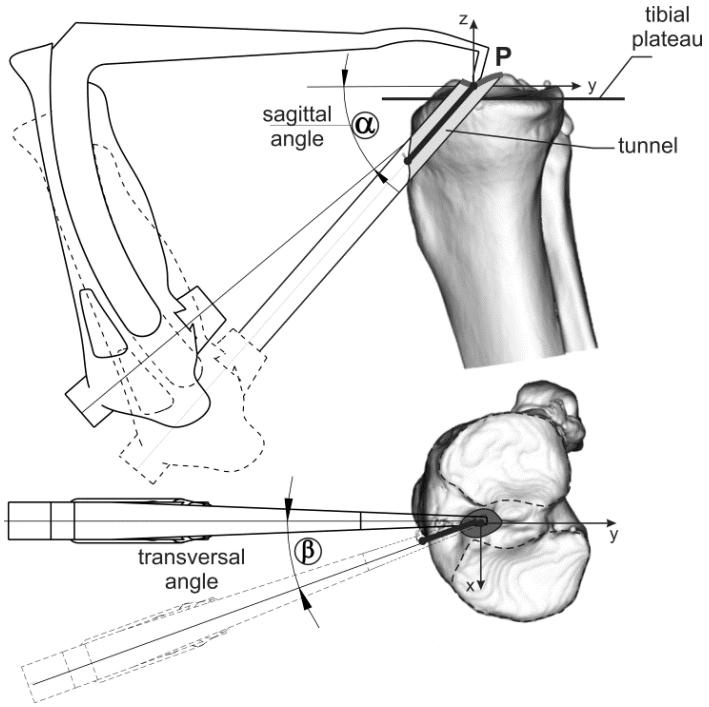


Fig. 2 – Graft insertion parameters.

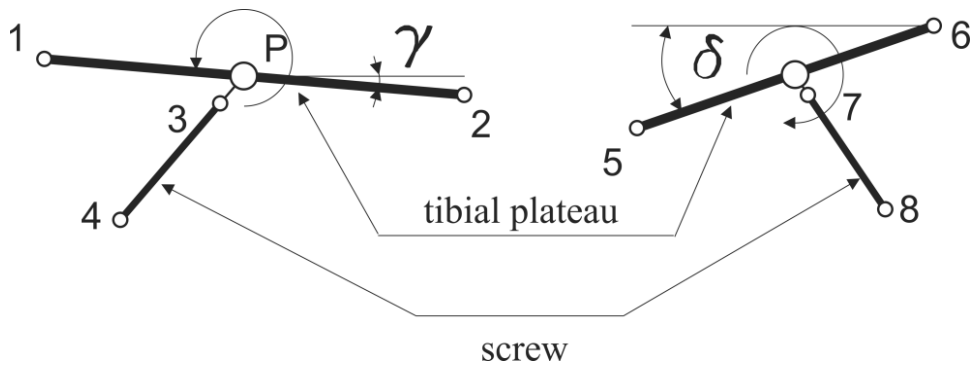


Fig. 3 – Defining the plane of the tibial plateau and tunnel axis.

the transversal angle was always different from 0º during the procedure. Such influences on the results of measurement are shown in Figure 5 as the projection values of sagittal, functioning as transversal angle, for the values from 0º to 90º. It can be seen in the figure that the sagittal angle for the value 0º was in its real size. The projected angle grows for other values; therefore, it was expected that X-ray images generated from the lateral view would show a bigger projected angle α_P between the graft and tibial plateau than the real drilling angle α. Consequently, inaccurate results were obtained, which may be misleading for a physician.

The mathematical procedure of sagittal angle calculation (α) based on the known projected sagittal angle in the lateral view (α_P) and transversal angle (β) shown in Figure 4 has been defined by the expressions (1)–(5).

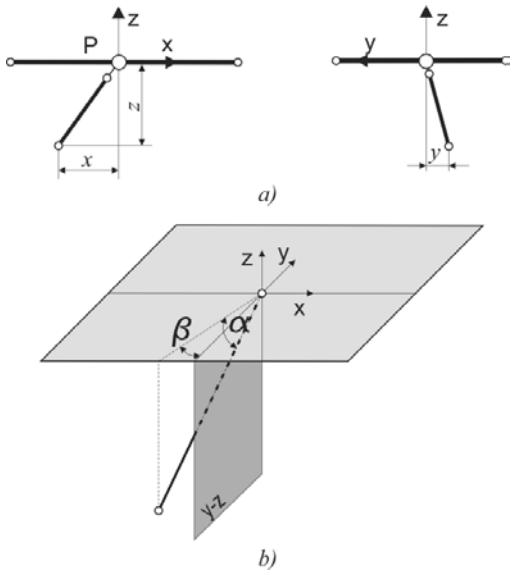


Fig. 4 – a) and b) – Principle of setting the selected parameters from X-ray images.
For details, see "Determination of graft angle" in Methods.

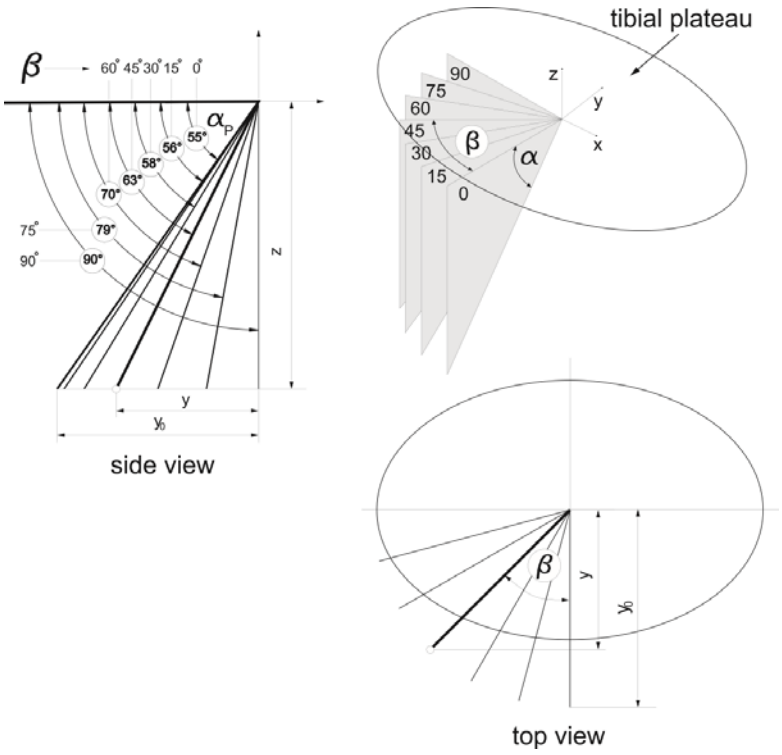


Fig. 5 – Correlation between sagittal angle α, transversal angle β, and projected sagittal angle α_P.
For details, see "Determination of graft angle" in Methods.

$\tan \alpha_p = z / y$ (1)
$y = y_0 \cos \beta$ (2)
$\tan \alpha = z / y_0$ (3)
$\tan \alpha = (z/1)/(y/\cos \beta) =$
$(z \cos \beta) / y = \tan \alpha_p \cos \beta$ (4)
$\alpha = \tan^{-1} (\tan \alpha_p \cos \beta)$ (5)

Structure of software

The development of adequate software that will confirm the success of the method for determining the graft insertion parameters comprises the formation of programming sections with the aim of applying the method described in the previous chapter, as well as the user interface that enables precise defining of specific tibial points, measurement of specific angles and their mathematical processing. For the automatization of these activities by applying the programming language C++ in the development environment Visual Studio 2010, a suitable programming solution has been developed. As verified software support for the development of image processing software in medicine and 3D graphics, Visualization Toolkit (VTK) library has been chosen for implementing the manipulation functions for graphic elements, as well as measurements of desired sizes in the images. VTK library possesses a large number of classes with functions that enable the following: loading and manipulating various scan formats; a high-resolution 3D display. Figure 6a shows a dialogue window, which is part of the user interface of the developed software solution with the parameters for selecting specific points; Figure 6b shows the output with the calculated parameters.

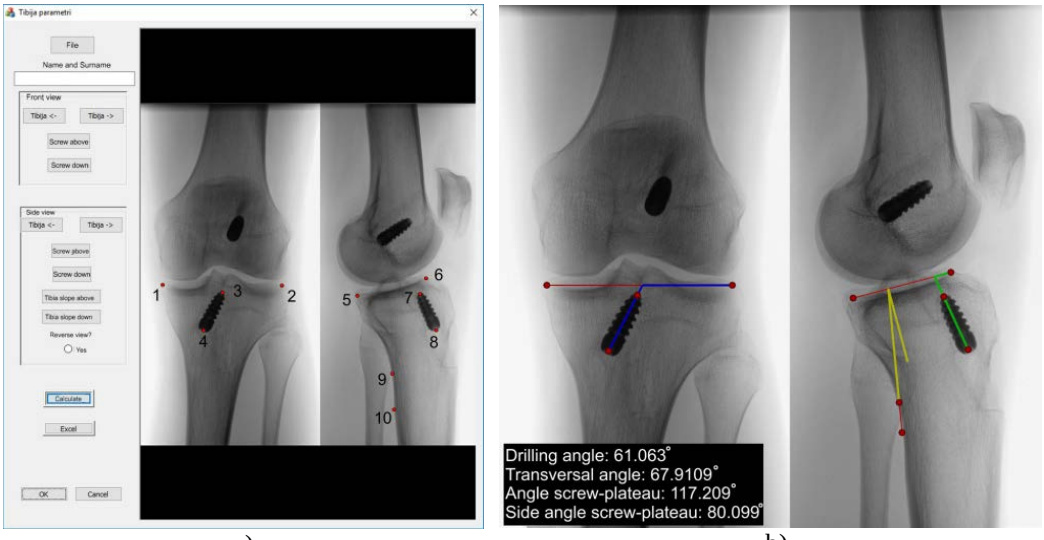
Figure 6a shows ten specific points in X-ray images chosen during the parameter calculation as follows: starting and end points of the tibial plateau in both images (points 1, 2, 5, and 6); starting and end graft points in both projections (points 3, 4, 7, and 8); points seen in the lateral image belonging to the tibial plateau angle (posterior tibial slope) (points 9 and 10). A created application enables detailed enlargement in the images during the selection of the appropriate points so that they can be selected as precisely as possible. Figure 6b shows the application output after calculation. It is a new image consisting of original images with the marked selected points, lines generated based on these points, and the calculated angles which are significant for the research. The angles are marked with the appropriate color, whereas the calculated values of the angles and their relationship at the tibial plateau in both projections from the graft intersection point with the tibial plateau are shown at the bottom of the image. The application enables exporting of the calculated values to the MS Excel table for further analysis and statistical processing.

Comparison of the obtained spatial drilling angle with CT images

To verify the newly developed software, the spatial determination of the graft position in the tibia was realized by processing standard X-rays (anterior-posterior and lateral projection) in 15 patients in whom the ACL reconstruction was performed. High-resolution digital radiograms were made using the Shimadzu Sonialvision Safire II. The same images were imported into the software developed for the analysis of graft spatial position in the tibia.

We used CT as a control method to determine the exact position of the graft in the tibia. The latest generation CT machine was used for this purpose (Siemens Somatom Emotion 16).

Tibial reconstruction using CT scans and applying 3D Doctor software has been performed to make a comparison of the drilling angle and carry out verification, with the result of achieving 3D models of the tibia and screws. Tibial and screw models generated in this manner are imported into the AutoCAD programming system, which enables precise measurement (with the unlimited possibility of image enlargement and measurement reading with more than ten decimal values) of the drilling angle.



a) b)

Fig. 6 – Developed application dialogue box display with selection parameters. For details, see "Structure of software" in Methods.

Results

A tunnel angle analysis has been carried out in a group of 15 patients with the aim of method verification for determining the tibial angle and functionality of the user interface of the programming system in the process of defining the specific points.

The obtained results are shown in Table 1. The first column represents the projected angle values measured based on the X-ray image, whereas the second and the third columns show a transversal drilling angle and the angle obtained using a developed computer program. A column with the values of the drilling angle based on the CT knee scan has been inserted into Table 1 to confirm the validity of the mathematical expression that describes the relationship between the real value of the drilling angle and the projected angle value.

Data in Table 2 shows that the drilling angle measured in CT scans, on average, is somewhat greater (59.07° ± 5.61°) than the angle measured by applying a developed application (58.65° ± 5.89°). CT drilling angle ranged from 45°– 68°, while during measurement using the application, it ranged from 42.94°– 67.93°. The median value was a half degree higher during measurement using the application. The distribution of measurement angles while measuring using both techniques is negatively asymmetrical, which indicates that the drilling angle values were higher than the mean value in a larger number of patients.

Wilcoxon test results (Z = -1.363; p = 0.173) showed that an error occurring during the determination of the spatial position of the tibial graft after ACL reconstruction by using a developed computer program has no statistical significance, and computer determination of tibial graft provides the same results as CT images.

Discussion

Although primary arthroscopic ACL reconstruction has reached until today a significant level of precision and is routinely performed, there are still a number of patients who are not completely satisfied with the outcome of surgical treatment and need reoperation. It is essential to find all the causes responsible for the unsatisfactory result of the primary reconstruction of this ligament in order to make a good preoperative preparation of the revision operation and thus reduce the number of complications to a minimum.

In order to achieve the projected postoperative outcome and knee stability during ACL reconstruction, it is necessary to position the graft properly and incorporate its ends into the created femoral and tibial tunnels ensuring isometry during movements. The most common reason for instability, even after surgery, is bad graft position, and as many authors say,

Table 1

Measurement results for 15 patients

Patient number	Projected angle, X-ray α_P[°]	Transversal angle, X-ray β[°]	Drilling angle, application α[°]	Drilling angle, computed tomography α[°]
1	71	45	65	65
2	68	48	58.9	58
3	81	67	67.9	68
4	68	46	59.8	61
5	74	60	60.2	61
6	49	36	42.9	45
7	71	59	56.2	55
8	60	45	50.8	51
9	79	65	65.3	64
10	70	51	60.0	61
11	71	53	60.2	61
12	69	52	58.1	59
13	67	49	57.1	58
14	73	58	60.0	61
15	68	49	58.4	58

Table 2

Descriptive statistics for the drilling angle α[°] measured using computed tomography (CT) and developed computer application

	CT	Application
Mean value	59.07	58.65
Standard deviation	5.61	5.89
Minimum	45	42.94
First quarter	58	57.09
Median value	61	59.82
Third quarter	61	60.2
Maximum	68	67.93
Wilcoxon test: Z = -1.363; p = 0.173		

improper femoral tunnel placement is a more common cause [14, 15–17]. However, improper tibial tunnel placement also results in recurrent instability.

Most surgeons who deal with this issue determine graft position (i.e., the tunnel where the graft is situated) in the tibia by measuring according to standard X-ray images, anterior-posterior and lateral projection, which is certainly not precise enough [12, 13], given that the results mainly depend on the morphology of bones and current position of the patient's extremities. The position of the graft can be most accurately determined from CT images, especially if 3D or multiplanar reconstruction is performed [16-18]. However, the high doses of radiation to which patients are exposed, as well as the cost of taking images, make it impossible to apply this method in everyday, routine clinical practice. Analysis of the position of the graft in the femur and tibia can also be performed via MRI unless metal lentils are used to fix the ends of the graft [15].

After ACL reconstruction, graft angle is one of the fundamental parameters in surgical outcome evaluation. The application of X-ray images in such determination, by its nature, provides plane (2D) scans with a relatively high error of orientation and positioning of the patient's knee, which results in an error occurring during graft angle measurement. The second method, using CT scans, is significantly more complex, more expensive [18], and less favorable for the patients due to its negative effects of radiation. Therefore, the application of X-ray images is the optimal method, accompanied by the formation of a suitable methodology for the determination of spatial graft angle.

The value of the transversal angle has a significant influence on the projection of the drilling angle; therefore, suitable mathematical transformation preceded by transversal angle evaluation from the X-ray image is necessary for the calculation of the real sagittal angle value.

Verification of the results obtained with the developed application was performed on a group of 15 patients, in whom, in addition to X-rays, CT images were also made. Tibial reconstruction using CT scans and applying 3D Doctor software has been performed to make a comparison of the drilling angle and carry out verification, with the result of achieving 3D models of the tibia and screws. A high level of automatization is achieved in this manner during this study phase, and subjective errors made by users while performing CT scan analysis are avoided. Tibial and screw models generated in this manner are imported into the AutoCAD programming system, which enables precise measurement of the drilling angle. The analysis of the obtained results showed that the drilling angle measured on CT images is, on average, slightly larger (59.07° ± 5.61°) than the angle measured by applying a developed application (58.65° ± 5.89°). This deviation from the results can be explained by the fact that during X-ray imaging, it is difficult to place the tibia in the ideal position so that the tibial plateau can be seen as a line.

A potential weakness of this experimental-clinical study lies in the fact that most authors regard CT or MRI evaluation as being more precise than radiographic measurement; therefore, clinical studies should include a larger number of ACL images made by using these techniques and introduce additional comparison criteria comprising the time of imaging and image reconstruction, price of diagnostics, etc.

One of the main aims of the study, part of which was presented in the paper, was to form a quick and economically efficient method for the determination of tibial graft position after surgery and the evaluation of reconstruction outcome based on that. A standard radiogram with appropriate software support was used as a basis for the created method. The obtained results are comparable with the measurement results using CT scans and suitable for clinical use on a daily basis from the standpoint of accuracy and usability.

Conclusion

Comparing the results obtained by measuring tibial tunnel angles after ACL reconstruction, one can reach a conclusion that the method of using X-ray images and software developed in accordance with them has the applicable value.

According to data obtained in this study, it has been concluded that this procedure may be applied to the determination of tibial tunnel placement as part of the preparation for revision surgery, i.e., in patients that still feel pain and knee instability after surgery.

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Nonsuicidal self-injury in a clinical sample of adolescents in Serbia

Nesuicidalno samopovređivanje u kliničkom uzorku adolescenata u Srbiji

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Abstract

Background/Aim. Nonsuicidal self-injury (NSSI) among adolescents is recognized as a significant public health concern. Adolescents with mental health problems are at an especially high risk of NSSI. The aim of the study was to assess the sociodemographic and clinical characteristics of patients with NSSI and the features of NSSI, as well as the impact of emotional reactivity and internalizing/externalizing problems on them. **Methods.** The observational study included a clinical sample of 111 adolescents treated at the Department of Child and Adolescent Psychiatry, Psychiatric Clinic at the University Clinical Center of Vojvodina, from March 2018 to October 2019. The adolescents were divided into two groups: group A comprised of adolescents who had at least one episode of NSSI in the last year, and group B comprised of adolescents who had no NSSI episode in their experience. The following clinical scales were used to examine the differences between adolescents from group A and group B: self-report questionnaires about emotional and behavioral problems (Youth Self-Report – YSR, Child Behavior Checklist – CBCL), emotional reactivity (Emotion Reactivity Scale – ERS), and suicidality (Self-Injurious Thoughts and Behaviors Interview – SITBI). **Results.** Group A contained a total of 58 adolescents older than the subjects of group B and was predominated by female participants. They had the first episode of NSSI at 13.05 and the last at 14.80 years. The most common methods of NSSI were cutting, scratching, and biting. Adolescents from group A had higher scores on ERS, higher scores on internalizing and externalizing problems, as well as higher total scores on YSR, but there was no difference between groups regarding scores on the CBCL scale. **Conclusion.** Due to the results obtained for emotional and behavioral problems, adolescents with NSSI should have a detailed psychiatric assessment, including social support, pharmacotherapy, and psychotherapy, to better understand NSSI and help them develop better coping skills.

Key words:
adolescent; adolescent psychiatry; psychopathology; self-injurious behavior.

Apstrakt

Uvod/Cilj. Nesuicidalno samopovređivanje (NSSP) među adolescentima prepoznato je kao značajan problem javnog zdravlja. Adolescenti sa problemima mentalnog zdravlja posebno su izloženi riziku od NSSP. Cilj rada bio je da se procene sociodemografske i kliničke karakteristike bolesnika sa NSSP i odlike NSSP, kao i uticaj emocionalne reaktivnosti i internalizacije/eksternalizacije problema na njih. **Metode.** Opservacionom studijom obuhvaćeno je 111 adolescenata koji su bili lečeni na Odeljenju za dečju i adolescentnu psihijatriju, Klinike za psihijatriju, Univerzitetskog Kliničkog centra Vojvodine, od marta 2018. godine do oktobra 2019. godine. Adolescenti su bili podeljeni u dve grupe: grupu A (adolescenti koji su imali najmanje jednu epizodu NSSP u poslednjih godinu dana) i grupu B (adolescenti koji nikada nisu imali NSSP). Za ispitivanje razlika između adolescenata grupe A i grupe B korišćeni su upitnici: o emocionalnim i bihejvioralnim problemima [Upitnik za samoprocenu adolescenata (*Youth Self-Report* – YSR) i Lista provere dečjeg ponašanja (*Child Behavior Checklist* – CBCL)]; o emocionalnoj reaktivnosti [Skala emocionalne reaktivnosti (*Emotion Reactivity Scale* – ERS)]; o suicidalnosti [Intervju o samopovređujućim mislima i ponašanju (*Self-Injurious Thoughts and Behaviors Interview* – SITBI)]. **Rezultati.** Grupu A činilo je ukupno 58 adolescenata koji su bili stariji od ispitanika grupe B i u njoj je dominirao ženski pol. Prvu epizodu NSSP imali su u uzrastu od 13,05 godina, a poslednju sa 14,80 godina. Najčešće metode NSSP bile su sečenje, grebanje i grickanje. Adolescenti grupe A postizali su više skorove: na ERS skali, u internalizovanju i eksternalizovanju problema kao i na ukupnom rezultatu na

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YSR skali. Nije bilo razlike između adolescenata grupe A i grupe B u odnosu na skorove postignute na CBCL skali. **Zaključak.** Adolescenti sa NSSP bi trebalo da imaju detaljnu psihijatrijsku procenu, koja bi uključivala socijalnu i psihološku podršku, farmakoterapiju i psihoterapiju, u cilju razumevanja i razvijanja veština prevladavanja NSSP.

Ključne reči:
adolescenti; psihijatrija, adolescentna; psihopatologija; samopovređivanje.

Introduction

Nonsuicidal self-injury (NSSI) is defined as deliberate, direct, self-inflicted destruction of body tissue without suicidal intent and outside socially sanctioned purposes [1]. The most common types of NSSI are cutting, burning, carving, scratching, picking wounds, head banging or punching, and hitting objects with hands or legs. Even though cutting was found to be the most common type of NSSI, most individuals use multiple methods of self-injurious behavior [2].

NSSI is a major public health concern as it has been linked to several poor outcomes and is found to place individuals at increased risk for suicide-related behavior [3]. The outset was noted in early adolescence, between the ages of 12 and 14 [4], peaking around mid-adolescence and declining toward late adolescence [1], but it can continue into adulthood [5]. It is estimated that 4% of adults present with NSSI [6]. A prevalence of 13% to 45% was found in the general adolescent population, with a greater clinical sample percentage, from 19% to 60% [7]. NSSI is mostly found in females. Gender differences were also found concerning the method of NSSI. Burning and self-hitting were more common in males while cutting and scratching were typically found in females [5, 8].

Even though the NSSI definition excludes suicidal intent, research indicates that individuals who present with NSSI are at greater risk of suicidal behavior [1]. That is why many research studies focus on examining the link between suicidal behavior and NSSI. Suicidal behavior and NSSI are increasingly being considered to exist along a continuum of self-harming behavior [9]. Compared to suicide attempts, NSSI occurs more frequently, implies minor physical harm [10], and absence of suicide intent [11]. Despite mutual differences, NSSI and suicide attempts often co-occur in both the general and clinical adolescent populations [6].

Many previous studies regarded NSSI as one of the characteristics of borderline personality disorder (PD). However, most recent study findings indicate a strong link between NSSI and different psychiatric disorders: depression, substance abuse disorder, posttraumatic stress disorder, generalized anxiety disorder, eating disorders (EDs), and other PDs [7, 12]. Adolescents who present with NSSI were found to have a greater incidence of both internalizing and externalizing problems [3]. In clinical practice, 87.6% of adolescents presenting with NSSI are also diagnosed with some psychiatric disorder [3].

NSSI is a maladaptive coping strategy with the purpose of emotional regulation. Its main function is to reduce or avoid difficult emotions, anxiety, sadness, or guilt [13–15]. Therefore, it makes sense that the most established affective factors related to NSSI are emotion dysregulation and emotional reactivity (ER) [15–17]. ER represents more trait-like vulnerability to heightened emotional experiences, whereas emotion dysregulation represents the inability to manage those experiences [18]. Those with high ER tend to experience emotions more intensely, for a longer time, and in response to a wider array of stimuli [16]. While a large amount of research has focused on emotion dysregulation and NSSI, much less work exists on the role of ER in NSSI engagement. The study conducted by Nock et al. [16], who used a combined sample of adolescents and young adults, found that ER was significantly related to the presence of NSSI. Additionally, ER statistically mediated the relation between psychopathology and self-injurious thoughts and behaviors [16].

The aim of the study was to assess the sociodemographic and clinical characteristics of patients with NSSI and the features of NSSI, as well as the impact of ER and internalizing/externalizing problems on them.

Methods

Participants

The study included 111 adolescents between 11 and 18 years of age, attending inpatient and outpatient services at the Department of Child and Adolescent Psychiatry, Psychiatric Clinic in University Clinical Center of Vojvodina, Serbia, sampled from March 2018 to October 2019. Participation in the study was voluntary and without financial compensation.

All participants were provided with a brief overview of the study aims and procedures along with written informed consent to participate in the research. Parental consent was required for participants under 18 years of age. Ethical approval was received from the institutional Ethical Board of the University Clinical Center of Vojvodina.

Instruments and procedure

All adolescents completed the following self-report questionnaires: The Youth Self-Report (YSR) [19] and Emotion Reactivity Scale (ERS) [16]. One of the parents or caregivers completed the Child Behavior Checklist (CBCL) [19]. Following the completion of YSR and ERS, adolescents were asked dichotomous questions (Yes/No) about self-harming (e.g., "Have you ever admittedly self-harmed without suicidal intent?"). For those participants who answered "Yes", a clinician-administered Self-Injurious Thoughts and Behavior Interview (SITBI) was performed [20]. The adolescents were

divided into two groups: the first group comprised of adolescents who had at least one episode of NSSI in the last year (i.e., group with NSSI), and the second group comprised of adolescents who had no NSSI episode in their experience (group without NSSI).

The YSR by Achenbach and Rescorla [19], consisting of 103 items, is one of the most widely used standardized self-report questionnaires for 11–18-year-olds that gives a dimensional description of behavioral/emotional problems that fall into four broad categories: Competence, Internalizing problems, Externalizing problems, and Total problem. Total competence includes activity, academic competence, and social competence. In addition to the total problem score, the problem items form nine narrowband syndrome scales ('withdrawn', 'somatic complaints', 'anxious/depressed', 'attention problems', 'thought problems', 'social problems', 'aggressive behavior', 'delinquent behavior', and 'self-destructive behavior/identity', which is a syndrome for boys only) and two broadband dimensions, 'internalizing' and 'externalizing'. Items that are not included in any of the eight syndromes are collected under the heading 'other problems', but they do not form a 'true' scale. The adolescent is asked to describe or rate their thoughts, emotions, and behaviors at present or in the previous six months on a three-point scale by circling 0 if the item or statement is not true, 1 if it is somewhat or sometimes true, and 2 if it is very true or often true. High scores on the problem items indicate more problems, and high scores on competence items indicate higher competence.

The CBCL is the parent report version of the Achenbach System of Empirically Based Assessment (ASEBA) [19]. Caregivers rated their children's behavior problems at ages 4–18. Item scores ranged from 0 (not true of the child) to 2 (very true or often true of the child). The internalizing scale sums 32 items loading onto three clinical syndrome scales: Withdrawn (9 items), Somatic complaints (9 items), and Anxious/Depressed (14 items). The externalizing scale sums 27 items from two clinical syndrome scales: Delinquent behavior (8 items) and Aggressive behavior (19 items).

The ERS is a self-report measure of emotional sensitivity, intensity, and persistence [16]. This study used a one-dimensional concept (i.e., total score) on this scale. The ERS has demonstrated good convergent, divergent, and criterion-related validity [16].

Self-injurious thoughts and behaviors were assessed using the SITBI [20], a structured clinical interview that assesses the presence, frequency, severity, age-of-onset, and other characteristics of a broad range of SITBI, including NSSI, suicide ideation, and suicide attempts. In the current study, only items that inquired about the presence of NSSI were included. The SITBI has strong interrater reliability, test-retest reliability over a 6-month period, and construct validity [20].

In addition to the above-listed measures, clinicians administered a specially designed semi-structured 9-item questionnaire that included factors found to be broadly associated with NSSI, such as demographic variables (age, sex), data related to suicide attempts (number of attempts, method, time), followed with a medical file review data about previous hospitalizations, psychopharmacotherapy treatments, heredity and diagnoses given by a clinician (child psychiatrist) according to the current International Classification of Diseases, Tenth Revision (ICD-10) criteria.

Permission to use the above-described measures was provided by the instrument authors.

Statistical analysis

Nonparametric χ^2 tests were used for statistical comparisons of the obtained data as well as independent samples *t*-test and multivariate analysis of variance (MANOVA). In situations where χ^2 was not statistically justified (frequency in cells lower than 5), we used contingency coefficient (C) as the measure of correlation.

Results

Sociodemographic characteristics

The sample comprised 64.8% of females and 35.2% of males. The average age was 14.83 (± 1.9) years. A total of 58 (52.3%) adolescents reported having at least one episode of NSSI in the last year, while 53 (47.7%) adolescents reported not having a single episode of NSSI in their experience.

Statistically significant differences were found regarding age in the NSSI group with older adolescents ($p = 0.05$). Moreover, there were differences regarding gender, with more females in the NSSI group ($p = 0.01$) (Table 1).

The average age for the first episode of NSSI in the current study sample was found to be 13.05 [standard deviation (SD) = 2.3] years and 14.80 (SD = 1.9) years for the last episode. The three most commonly noted methods of NSSI were cutting (81%), scratching (65.5%), and biting (62%). Overall, 19% of the adolescents in our sample reported using only one method, whereas 68% used more than three different methods of NSSI. Results indicate that 51.7% of adolescents had more than five episodes of NSSI in their lifetime, 27.6% in the last year, and 8.6% in the last month.

Table 1

Comparison between the nonsuicidal self-injury (NSSI) group and the non-NSSI group in sociodemographic features

Sociodemographic features	NSSI (n = 58)	Non-NSSI (n = 53)	Statistics	*p*
Age (years), mean (SD)	15.2 (1.732)	14.5 (2.099)	-1.972*	0.05
Gender, female (%)	75.9	52.8	6.446#	0.01

SD – standard deviation.
***= *t*-test; # = χ^2 test.**

Most (65%) adolescents in the NSSI group reported never receiving any treatment for NSSI. Similarly, 53% admitted that, at the time of assessment, they did not avail of mental health services for NSSI but rather sought help for other mental health-related reasons. In the NSSI group, 81% reported their parents/caregivers were aware of their self-harming behavior. Results show that 65% of adolescents, who present with NSSI, did not search for NSSI information on the internet, and 77% reported they did not access NSSI-related websites.

Psychiatric diagnosis, heredity, suicidal attempts, and NSSI

Regarding psychiatric diagnosis in line with ICD-10 criteria, results indicate lower intensity positive correlation between diagnosis and presence/absence of NSSI. The results show that in the group with NSSI, there were more adolescents with the following diagnoses: F1, F3, F4, and F6, and fewer participants with diagnoses F2, F5, F8, and F9 (Table 2). It was found that 46.3% of adolescents have a positive hereditary predisposition for psychiatric disorders. However, the results indicate no statistically significant differences between adolescents with NSSI and those without NSSI (Table 2).

The current study sample comprised adolescents in inpatient (66.7%) and outpatient (33.3%) treatment. The results show no statistically significant difference regarding the presence of NSSI. Variable history of previous hospitalizations was not found to be statistically significant (Table 2).

In our clinical sample, 17.1% of adolescents were found to have had a suicidal attempt in the past, and 6.3% reported having multiple suicidal attempts. A significant correlation (p = 0.000) was noted between adolescents with and those without NSSI concerning the suicidal attempt variable (Table 3). The group with NSSI had more adolescents who attempted suicide compared to the group without NSSI. In addition to that, results indicate that adolescents with NSSI reported multiple suicidal attempts (p = 0.019) compared to adolescents without NSSI.

Difference between groups on ERS, YRS, and CBCL

Table 4 shows a statistically significant difference between groups regarding ER. Those adolescents with NSSI had higher scores on ERS (p = 0.000).

The results of MANOVA indicate a significant difference between groups regarding internalizing, externalizing, and total scores on YSR (p = 0.000). Statistically, a

Table 2

Comparison between the nonsuicidal self-injury (NSSI) group and the non-NSSI group in psychiatric diagnosis and comorbidities

Parameter	NSSI (n = 58)	Non-NSSI (n = 53)	Statistics	*p*
Hospitalization (yes) (%)	32.7	34.0	0.018#	0.893
Psychiatric heredity (yes) (%)	46.3	28.8	3.433#	0.062
Clinical psychiatric diagnosis, ICD-10 (%)			0.334*	0.052
F1 (substance use disorder)	3.4	0		
F2 (psychotic disorders)	3.4	9.4		
F3 (affective disorders)	6.9	1.9		
F4 (anxious disorders)	50	39.6		
F5 (eating disorders)	0	7.5		
F6 (personality disorders)	15.5	7.5		
F8 (pervasive disorders)	0	1.9		
F9 (conduct disorder, ADHD)	20.7	32.1		
Comorbidity (%)			0.104*	0.544
no	74.2	75.5		
one diagnosis	17.2	20.8		
two diagnoses	8.6	3.8		

ICD-10 – International Classification of Diseases, Tenth Revision; ADHD – attention deficit hyperactivity disorder.

 # = χ^2 test; *= contingency coefficient (C).

Table 3

Comparison between the nonsuicidal self-injury (NSSI) group and the non-NSSI group in suicidal attempts and methods

Parameter	NSSI (n = 58)	Non-NSSI (n = 53)	C	*p*
Suicidal attempt (yes) (%)	29.3	3.8	0.321	0.000
Suicidal attempts over a lifetime (%)			0.346	0.002
last month	5.2	3.8		
last year	13.8	0		
more than a year	10.3	0		
Suicide method (%)			0.324	0.005
intoxication	20.7	3.8		
cutting	6.9	0		
other	1.7	0		

C – contingency coefficient.

Table 4

Differences between the nonsuicidal self-injury (NSSI) group and the non-NSSI group on Emotion Reactivity Scale (ERS), Youth Self-Report (YSR), and Child Behavior Checklist (CBCL)

Parameter	NSSI (n = 58)	Non-NSSI (n = 53)	*t*- test (*F-test)	*p*
ERS (mean)	54.4	33.7	5.979	0.000
YSR (T score mean)				
internalizing	70.17	57.56	29.784*	0.000
externalizing	63.64	52.63	28.933*	0.000
total	69.03	55.70	44.398*	0.000
CBCL (T score mean)				
internalizing	67.22	65.58	0.807*	0.371
externalizing	62.31	58.79	2.855*	0.094
total	66.07	63.58	2.307*	0.132

significant difference was found in relation to the mean T score on three dimensions of the YSR scale: internalizing, externalizing, and total problems. Those with NSSI have higher scores on all three dimensions (Table 4).

The results of MANOVA show no significant difference between groups regarding internalizing, externalizing, and total scores on the CBCL scale ($p = 0.423$). Likewise, no significant difference was found regarding the mean T score on all three dimensions: internalizing, externalizing, and total problems on the CBCL scale (Table 4).

Discussion

The aim of the research was to investigate the phenomenological characteristics of NSSI as well as similarities and differences between two groups of adolescents within a clinical population where the main distinguishing factor was the presence/absence of NSSI.

More than half (52.3%) of the participants in our clinical sample had NSSI at least once in the last year. These findings have been confirmed in previous research studies indicating that the frequency of NSSI in the clinical population goes up to 60% [7, 21–23]. The current study results align with the results obtained by Kaess et al. [24], who found that the frequency of NSSI in a clinical sample with one NSSI is 60%, and for repetitive NSSI is 50%. Similar results were found in an Austrian study where 50.8% of inpatient adolescents between 11 and 17 years of age had at least one NSSI [23].

Our research also confirmed earlier findings of greater frequency of NSSI in females that is particularly significant in a clinical sample of adolescents [8, 14, 22, 23]. One of the explanations for this could be that male adolescents rarely seek help regarding their psychological difficulties due to fear of stigmatization. Furthermore, it is well known that depression and anxiety are strongly correlated with NSSI and that those disorders are more prevalent among females who ask for help than among males. That was evident in our clinical sample, which comprised 64.8% of females. Likewise, some types of NSSI, such as punching the wall or objects or hitting their body against the wall, are regarded as physical aggression directed outward and are not registered as NSSI. All this can have an impact on poor cognition, diagnosing NSSI, and lack of intervention for NSSI in males [25].

Results indicate a significant difference between groups regarding age, where the group without NSSI had an average of 14.5 years and the group with NSSI 15.2 years. This result can be explained by longitudinal research findings, which indicated that NSSI reaches "the peak" during middle adolescence, i.e., 15–16 years of age [1].

Like other research findings [9, 26], cutting, scratching, and biting have been found as the most frequent method of NSSI in our sample. Moreover, 68% of participants have been found to use multiple NSSI methods [2, 13, 27], and multiple NSSI method use indicates higher suicidal risk, which is also in line with previous research findings [28].

Results of our study confirm an increased risk of attempting suicide and repeated suicide attempts for adolescents who present with NSSI, which is in line with numerous studies [3, 29]. Over the course of research that was conducted in the past two decades, it has become clear that NSSI and suicide attempts go side by side; if one form of behavior exists, there is a greater possibility that the second form will be present as well.

The link between NSSI and clinical diagnosis according to ICD-10 is also evidenced in our research. We found that the NSSI group had a higher number of individuals with the following diagnoses: substance addiction, mood disorder, anxiety disorder, and PD; fewer participants presented with psychotic disorders, ER, and attention deficit hyperactivity (ADHD). Most of these results are in line with what was expected based on the review of previous research findings [7, 12, 23], exclusive of the results concerning EDs. Various research findings indicate a strong link between NSSI and ED (mainly *bulimia nervosa*), which can be explained by the common risk factors, including emotional dysregulation and high ER [30]. A possible explanation could be that there were fewer participants with an ER in our sample.

Various research findings have, like ours, confirmed the link between externalizing and internalizing problems and NSSI [7, 31]. For instance, Nock et al. [3] found that 62.9% of adolescents with NSSI presented with an externalizing disorder, and 51.7% presented with an internalizing disorder. Particularly interesting are the findings of our research, which indicate significant differences between groups regarding the YSR scale (more internalizing and externalizing problems in the NSSI group). However, this difference between groups was not found on CBCL. One of the explanations for this re-

sult could be that NSSI is an expression of subjective distress that cannot be registered easily by others. Likewise, results highlight the importance of adolescent self-report in the detection of NSSI among clinical population adolescents.

Even though more than 10 years ago, Nock et al. [16] created the ERS and found that the construct of ER can partly explain the link between NSSI and psychopathology, this research field is lacking studies aiming to investigate ER in a clinical sample of adolescents. Our research clearly indicates the association between ER and NSSI, as we have found that the group with NSSI achieved significant scores on the ERS. Similar results were found in a study that included a smaller and slightly older sample of adolescents (average age 20 years) [32]. Thus far, there is not enough evidence indicating whether her psychopathology is the cause or a consequence of high ER. However, the evidence does suggest that high ER among individuals who present with psychopathology can lead to NSSI [16].

Despite the strengths of the results, the findings of this study should be viewed in the context of its limitations. The limitations of this study are the following: (1) relatively small sample size; (2) combined with other research that includes self-report questionnaires, results could be influenced by the fact that participants might have answered in a socially desirable manner and, in addition to this, there is a possibility of memory distortion; (3) this is a cross-sectional study, and only longitudinal study would enable monitoring of these variables over time.

Conclusion

A high level of NSSI is present in the clinical sample of adolescents. Girls were found to be more likely to present with NSSI, and the most common methods are cutting and scratching with the high level of multiple NSSI method use. Between groups of adolescents who present with NSSI and those without NSSI, significant differences were noted in the variables gender, clinical diagnosis, and previous suicidal attempts. Adolescents with NSSI have more prominent internalizing and externalizing problems and a higher degree of ER. Even though these two clinical groups were different regarding many factors, our study was unable to explain the existence of two distinct groups within a vulnerable clinical group of adolescents with mental health problems, nor was it able to define their potential characteristics. That was recognized as a gap that would warrant further research. The practical importance of our study is that NSSI was found to be a signal for many other associated problems in a clinical sample of adolescents and outlines the need for a more complex approach to treatment among clinicians.

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Transobturator tape surgery experience: urodynamic evaluation of 220 patients in a single tertiary center in Turkey

Iskustvo u hirurškom lečenju primenom transopturatorne trake: urodinamska procena 220 bolesnika u tercijarnom centru u Turskoj

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Abstract

Background/Aim. Stress urinary incontinence (SUI) is the involuntary leakage of urine after increased intraabdominal pressure, and it causes a significant public health problem by reducing the quality of life, causing sexual dysfunction, and increasing the cost of care due to increased morbidity. The aim of the study was to investigate the intra- and postoperative results and complication rates of the transobturator tape (TOT) procedures used for SUI treatments in a tertiary center located in central Turkey. **Methods.** This prospective study analyzed a total of 220 patients undergoing TOT procedures for SUI. The demographic and clinical characteristics, preoperative and postoperative cystometry values, and operative outcome parameters of the study participants were analyzed. **Results.** While no significant difference was noted between the preoperative and postoperative periods with respect to residual volume (27.09 ± 8.51 mL vs. 26.01 ± 3.51 mL, p = 0.125), there were significant differences in terms of the first urinary urge (142.61 ± 20.25 mL vs. 145.64 ± 20.91 mL, $p < 0.001$), maximum bladder capacity (423.70 ± 38.43 mL vs. 402.32 ± 39.46 mL, $p < 0.001$), the Q-tip angle (45.54 ± 5.33^0 vs. 43.81 ± 6.15^0, $p = 0.001$), the maximum flow rate (37.65 ± 11.54 mL/s vs. 24.38 ± 9.26 mL/s, $p < 0.001$), average flow rate (19.92 ± 9.64 mL/s vs. 14.77 ± 8.71 mL/s, $p < 0.001$), the number of urinations in the daytime (7.29 ± 1.35 vs. 6.58 ± 1.29, $p < 0.001$), and the number of urinations at nighttime (1.48 ± 1.01 vs. 0.92 ± 0.83, $p < 0.001$). **Conclusion.** The TOT procedure improves the quality of life of SUI patients and can reduce morbidity. Nevertheless, additional studies are needed to corroborate our findings and determine the long-term effects.

Key words:
female; suburethral slings; surgical procedures, operative; urinary incontinence, stress.

Apstrakt

Uvod/Cilj. Urinarna stres inkontinencija (USI) je nevoljno ispuštanje urina posle povećanja intraabdominalnog pritiska što predstavlja značajan zdravstveni problem, smanjuje kvalitet života, uzrokuje seksualnu disfunkciju i povećava troškove nege zbog povećanog morbiditeta. Cilj rada bio je da se ispitaju intra- i postoperativni rezultati i stopa komplikacija u procedurama u kojima se primenjuje transopturatorna traka (TOT) u lečenju USI, u tercijarnom zdravstvenom centru u centralnoj Turskoj. **Metode.** Prospektivnom studijom ispitana su 22 bolesnika podvrgnuta proceduri u kojoj se primenjuje TOT u lečenju USI. Analizirane su demografske i kliničke karakteristike, preoperativne i postoperativne vrednosti cistometrije i parametri ishoda operativnog lečenja. **Rezultati.** Nije utvrđena značajna razlika između preoperativnog i postoperativnog perioda u odnosu na rezidualni volumen (27,09 ± 8,51 mL vs. 26,01 ± 3,51 mL, p = 0,125), a nađena je značajna razlika u pogledu prvog poziva na mokrenje (142,61 ± 20,25 mL vs. 145,64 ± 20,91 mL, $p < 0,001$), maksimalnog kapaciteta mokraćne bešike (423,70 ± 38,43 mL vs. 402,32 ± 39,46 mL, $p < 0,001$), Q-tip ugla (45,54 ± 5,33^0 vs. 43,81 ± 6,15^0, p = 0,001), maksimalnog protoka urina (37,65 ± 11,54 mL/s vs. 24,38 ± 9,26 mL/s, $p < 0,001$), prosečnog protoka urina (19,92 ± 9,64 mL/s vs. 14,77 ± 8,71 mL/s, $p < 0,001$), broja mokrenja (BM) u toku dana (7,29 ± 1,35 vs. 6,58 ± 1,29, $p < 0,001$) i BM u toku noći (1,48 ± 1,01 vs. 0,92 ± 0,83, $p < 0,001$). **Zaključak.** Procedura u kojoj se primenjuje TOT poboljšava kvalitet života bolesnika sa USI i može smanjiti morbiditet. Neophodna su dodatna istraživanja da bi se potvrdili rezultati ove studije i utvrdili dugoročni efekti.

Ključne reči:
žene; trake, suburetralne; hirurgija, operativne procedure; inkontinencija, urinarna, stress.

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Introduction

Stress urinary incontinence (SUI), which can cause social and hygienic problems, affects 14–35% of women [1, 2]. It is the involuntary leakage of urine after increased intraabdominal pressure in certain situations, such as exercise, sneezing, coughing, and laughing, without bladder detrusor muscle contraction [2, 3]. SUI causes a significant public health problem by reducing the quality of life, causing sexual dysfunction, and increasing the cost of care due to increased morbidity [4, 5]. Old age, obesity, interventional vaginal delivery, positive family history, smoking, diabetes mellitus, stroke, menopause, genitourinary surgery, cognitive impairment, and dementia are all risk factors for urinary incontinence [6, 7].

Urethral hypermobility and intrinsic sphincteric deficiency are the two main mechanisms involved in the etiopathogenesis of SUI [8, 9]. In urethral hypermobility cases, there is insufficient support from the suburethral and vaginal connective tissue and pelvic floor muscles to the bladder neck, which results in increased intraabdominal pressure while coughing or sneezing, or in the loss of connective tissue and pelvic muscular strength due to a particularly interventional vaginal delivery [8]. In intrinsic sphincteric deficiency cases, neuromuscular damage occurs due to pelvic or incontinence surgery, aging, and menopause [9].

The aim of the SUI treatment is to relieve the symptoms and improve the quality of life of the female patient. Both conservative and surgical options are available for the treatment of SUI. The conservative approach includes the elimination of certain factors that cause SUI, such as smoking, the use of certain types of pessaries, pharmacological therapy, and physiotherapy. Kegel exercises are the primary physiotherapy treatment for SUI [10–12].

Marshall et al. [13] first described the simple vesicourethral suspension technique as a surgical treatment for SUI. Subsequently, in 1961, Burch [14] developed a technique that included hanging the vesicourethral tissues on Cooper's ligament. Instead of hanging the vesicourethral tissues, Tanagho [15] suggested a colpocystourethropexy technique to prevent postoperative voiding dysfunction. However, suburethral sling procedures are the basis of the surgical treatment of SUI. These procedures consist of either mid-urethral or bladder neck slings. The slings placed in this area do not always correct SUI, can cause bladder hyperactivity or even bladder obstruction occasionally, and produce a bladder emptying difficulty. Mid-urethral slings include retropubic mid-urethral slings (e.g., tension-free vaginal tape), transobturator tape (TOT) mid-urethral slings, and single-incision mid-urethral slings. Bladder neck slings, also called proximal urethral slings, are fixed to Cooper's ligament or anterior rectus muscle fascia via an abdominal or vaginal incision [16]. The mid-urethral sling procedure was performed for the first time in 1996 using a mesh through the retropubic space with the aid of trocars [17]. In 2001, TOT slings were introduced by Delorme in order to avoid retropubic insertion complications, such as bladder perforations, vascular injuries, and bowel injuries [18].

The aim of this study was to compare intra- and postoperative results and complication rates with the TOT procedures used for SUI treatments in a tertiary center located in central Turkey.

Methods

This prospective cohort study included 220 TOT procedures carried out between January 1, 2015, and December 31, 2020, at the Gynecology Department of Konya Education and Research Hospital, a tertiary institution serving as a referral center for Konya and middle Anatolia in Turkey. This study was approved by the institution's local Ethics Committee from September 30, 2016 and written informed consent for all of the gynecological operations was obtained. The ethical principles for medical research involving human subjects stipulated in the 18th World Medical Association Declaration of Helsinki were applied. Because of the learning curve, the surgical procedures were all performed by two senior consultant gynecologists.

The inclusion criteria for this procedure were patients with symptomatic SUI who had occult SUI and who had no contraindications for lithotomy. We excluded the TOT procedures used for patients with urge incontinence, active urinary tract infections, current pregnancies, and anticoagulant treatments to prevent the risk of hematoma formation.

Presurgical preparation

Each patient undergoing a TOT procedure was admitted to the outpatient clinic of the hospital one day before their scheduled operation. A standard preoperative assessment (cell blood count, coagulation tests, and electrocardiography) was performed, and prophylactic intravenous antibiotics (1 g of cefazolin) were administered to all patients as premedication by a senior nurse approximately 30 min before the surgery. A bladder catheter was inserted during the surgical procedure and was withdrawn 6–8 hrs after the patient's mobilization.

TOT surgical procedure

After the patient was placed in the dorsal lithotomy position, disinfection and sterile coverage were performed. Local anesthetic with epinephrine was injected paraurethrally into the lower parts of the vaginal wall. The TOT procedure was carried out externally to internally, as described by Delorme [18]. Polypropylene mesh tape covered with a plastic sheath and helical trocars (I-STOP; CL Medical, Lyon, France) were used for this procedure. The duration of the TOT procedure was measured from the time of administering the local anesthetic to the moment of closing sutures, and the duration was recorded.

Data analysis

The sociodemographic characteristics included age, body mass index (BMI), gravity, parity, duration of symp-

toms (years), episiotomies performed, Cesarean sections performed, number of patients in menopause, menopausal period (years), systemic diseases, hypertension, diabetes, goiter, chronic obstructive pulmonary disease, educational level, economic status, residence, smoking status, alcohol consumption, caffeine consumption, cell phone usage, and drug abuse. The preoperative and postoperative (at the first week, third month, sixth month, and first year) cystometry values regarding the first urinary urge, maximum capacity, Q-tip angle, residual volume, maximum flow rate (Qmax) (mL/s), average flow rate (Qave) (mL/s), number of urinations during the daytime, and number of urinations at nighttime were evaluated. In addition, the operative outcome parameters in terms of surgical success, operation duration (min), preoperative hemoglobin (Hb), postoperative Hb, hospital stay, and complications were also checked. The patients were called for a control examination every three months in the first year after surgery. The absence of urine incontinence during stress maneuvers in the pelvic examination postoperatively in the third month was considered a success of the surgery.

Statistical analysis

We used the Statistical Package for the Social Sciences version 15 (SPSS Inc., Chicago, IL, USA) for the statistical analyses. The distributions of all continuous variables for the normal or nonnormal distributions were tested using the Kolmogorov-Smirnov test. The homogeneity of the variances was evaluated using the Levene test. Metric discrete and continuous variables were expressed as the mean ± standard deviation where applicable. The nominal data were expressed as the number of cases and percentages. Although the normally distributed variables between the groups were compared using a paired-sample *t*-test, the Wilcoxon test was applied to the nonnormally distributed variables. A *p*-value of < 0.05 was considered statistically significant.

Results

Seventy-nine of the 299 patients evaluated between January 1, 2015, and December 31, 2020, were not eligible for the study and were thus excluded (urge incontinence, urinary tract infection, current pregnancy, and/or anticoagulant treatment). The remaining 220 patients were analyzed, as shown in Figure 1. All of the patients were observed from the start of the surgery until they were discharged from the hospital.

The demographic and clinical characteristics of the study participants are presented in Table 1. The mean age of the patients was 53.87 ± 6.22 years, and the BMI was 30.95 ± 3.13 kg/m^2 at the end of the study. The systemic disease incidence was 128, and the mean duration of symptoms was 6.20 ± 2.72 years.

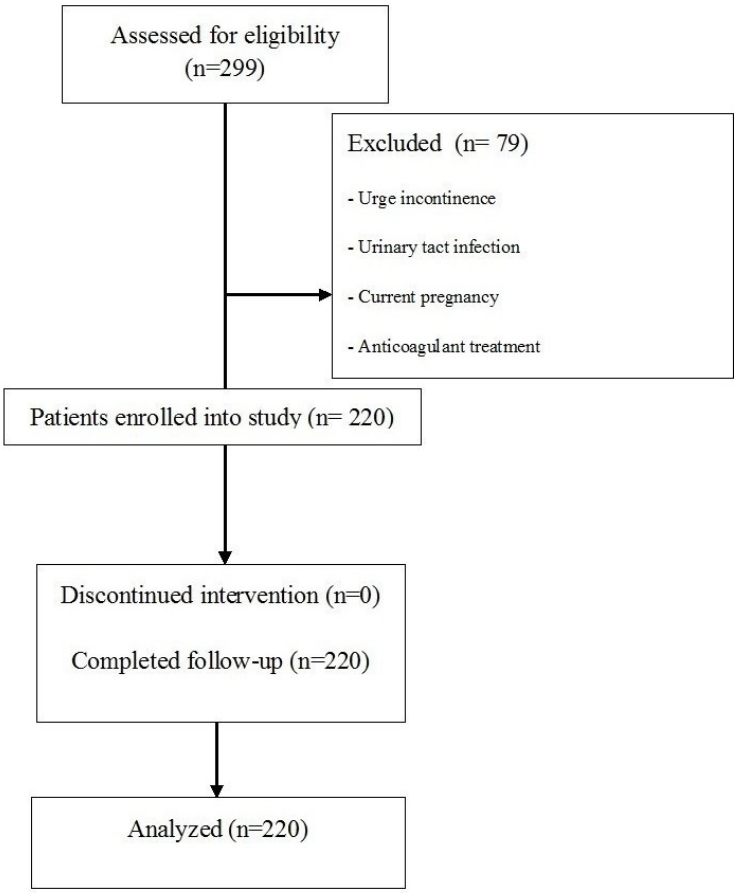

Fig. 1 – Enrollment and follow-up of the study subjects.

Table 1

Demographic and clinical characteristics of the study participants (n = 220)

Parameter	Values
Mean age (years)	
mean ± SD	53.87 ± 6.22
median (min-max)	53.00 (45.0–65.0)
BMI (kg/m^2)	
mean ± SD	30.95 ± 3.13
median (min-max)	31.00 (24.0–38.0)
Gravity	
mean ± SD	4.18 ± 1.47
median (min-max)	4.00 (2.0–7.0)
Parity	
mean ± SD	3.23 ± 1.16
median (min-max)	3.00 (1.0–6.0)
Duration of symptoms (years)	
mean ± SD	6.20 ± 2.72
median (min-max)	6.00 (2.0–15.0)
Patients with performed episiotomy, n (%)	148 (67.3)
Patients who delivered via Cesarean section, n (%)	21 (9.5)
Patients in the menopausal period of life, n (%)	155 (70.5)
Menopausal period (year)	
mean ± SD	7.61 ± 4.40
median (min-max)	7.00 (1.0–17.0)
Systemic diseases, n (%)	
hypertension	42 (19.1)
diabetes mellitus	21 (9.5)
goiter	12 (5.5)
chronic obstructive pulmonary disease	16 (7.3)
hypertension + diabetes mellitus	16 (7.3)
hypertension + goiter	11 (5.0)
hypertension + chronic obstructive pulmonary disease	6 (2.7)
diabetes mellitus + goiter	4 (1.8)
Education level (%)	
illiterate	8 (3.6)
primary or secondary school	147 (66.9)
high school	43 (19.5)
university	22 (10.0)
Economic status, n (%)	
Lower level (< 5,000 dollars/year)	60 (27.3)
Intermediate level (5,000–10,000 dollars/year)	122 (55.4)
High level (> 10,000 dollars/year)	38 (17.3)
Residence lives, n (%)	
village	73 (33.2)
town	67 (30.5)
city	80 (36.4)
Smoking status, n (%)	84 (38.2)
Alcohol consumption, n (%)	16 (7.3)
Caffeine consumption, n (%)	41 (18.6)
Cell phone usage, n (%)	183 (83.2)
Drug abuse, n (%)	7 (3.2)

BMI – body mass index; SD – standard deviation; min – minimum; max – maximum.

The preoperative and postoperative cystometry values are shown in Table 2. While no significant difference was noted between the preoperative and postoperative periods with respect to residual volume, there were significant differences in terms of the first urinary urge ($p < 0.001$), maximum bladder capacity ($p < 0.001$), the Q-tip angle ($p = 0.001$), Qmax ($p < 0.001$), Qave ($p < 0.001$), number of urinations during the daytime ($p < 0.001$), and number of urinations at nighttime ($p < 0.001$).

The operative outcome parameters of the patients are summarized in Table 3. The mean operation duration was 18.43 ± 3.98 min, the preoperative mean Hb level was 11.20 ± 0.94 g/dL (normal range 12–16 g/dL), the postoperative mean Hb level was 10.97 ± 0.95 g/dL, and the mean hospital stay was 1.02 ± 0.13 days. While there was no bladder, urethral, bowel, or vascular injury or pelvic hematomas during the surgical procedures, there was groin pain in 4 patients, urinary tract infections in 3 patients, vaginal injuries in 2

Table 2

Preoperative and postoperative cystometry values at first year

Parameter	Preoperative	Postoperative	*p*-value
First urine urge (cc)	142.61 ± 20.25	145.64 ± 20.91	< 0.001
Maximum bladder capacity (cc)	423.70 ± 38.43	402.32 ± 39.46	< 0.001
Q-tip angle	45.54 ± 5.33	43.81 ± 6.15	0.001
Maximum flow rate (mL/s)	37.65 ± 11.54	24.38 ± 9.26	< 0.001
Average flow rate (mL/s)	19.92 ± 9.64	14.77 ± 8.71	< 0.001
Residual volume (cc)	27.09 ± 8.51	26.01 ± 3.51	0.125
Number of urinations in daytime	7.29 ± 1.35	6.58 ± 1.29	< 0.001
Number of urinations at nighttime	1.48 ± 1.01	0.92 ± 0.83	< 0.001

cc – cubic centimeters.

Table 3

Operative outcome parameters of the patients

Parameter	Values
Success of surgery, n (%)	211 (95.6)
Duration of operation (min)	
mean ± SD	18.43 ± 3.98
median (min-max)	18.00 (13.0–23.0)
Preoperative Hb (g/dL)	
mean ± SD	11.20 ± 0.94
median (min-max)	10.70 (9.0–13.0)
Postoperative Hb (g/dL)	
mean ± SD	10.97 ± 0.95
median (min-max)	10.60 (9.0–13.0)
Hospital stay (days)	
mean ± SD	1.02 ± 0.13
median (min-max)	1.00 (1.0–2.0)
Complications, n (%)	15 (6.8)
Groin pain	4 (1.8)
Urinary tract infection	3 (1.3)
Vaginal injury	2 (0.9)
Dyspareunia	2 (0.9)
Urine retention	2 (0.9)
Mesh erosion	2 (0.9)

Note: Normal range for hemoglobin (Hb) is 12–16 g/dL.
SD – standard deviation; min – minimum; max – maximum.

patients, dyspareunia in 2 patients, urine retention in 2 patients, and mesh erosion in 2 patients, and all of them were observed in the early stage. The patients were given medical treatments for groin pain, urinary tract infections, and dyspareunia, and the vaginal lacerations were primarily repaired. The mesh erosions were followed-up because they were asymptomatic. The urine retention cases were treated with bladder catheterizations for one week on average, and then, the symptoms disappeared. The success of the surgery was determined at 95.6% in the third month. No complications were observed during the long-term follow-up.

Discussion

In this study, we assessed the results of the TOT procedures used for SUI treatments and compared the intra- and postoperative results and complication rates. Significant differences were seen in terms of the first urinary urge, maximum bladder capacity, the Q-tip angle, Qmax, Qave, number of urinations during the daytime, and number of urinations at nighttime.

With the increased life expectancy, SUI remains a major health problem. SUI is generally more common in women in the middle to older age groups and during the postmenopausal period. The average age of the women who underwent TOT procedures due to SUI ranged from 46 to 58 years in various studies [10, 19–21], and in our study, the average age was 53.87 ± 6.22 years. Additionally, 155 (70.5%) participants were in the postmenopausal period. The reason that SUI is more common in the menopausal period may be due to the rapid decrease of the higher estrogen levels during the reproductive period with the onset of menopause. These results were similar to those from the study conducted by Ulubay et al. [20].

The TOT procedure has a very short operative time and hospitalization stay compared to the other sling procedures. In various studies, the operation time varied between 12 and 23 min [22, 23]. In accordance with the literature, the operation duration in our study was 18.43 ± 3.98 min and the hospitalization time was 1.02 ± 0.13 days.

A woman with normal bladder function can fully discharge more than 80% of the bladder volume, with a post-

void residual volume of less than 50 cc [24]. There was no significant difference between the participants in terms of the residual volumes during the preoperative and postoperative periods. In sling procedures, postoperative edema and painful urination may cause retention. If this retention lasts longer than one week, the sling tape should be loosened using the same vaginal incision line as soon as possible [25, 26]. Transient urinary retention was observed in 2 (0.9%) patients following the surgical procedure. These patients underwent bladder catheterizations for an average of one week, and the urine retention symptoms disappeared.

The most important characteristic that separates a TOT procedure from other sling operations is its low complication rate. While voiding difficulty is the most prominent complication in the Burch [14] procedure, groin pain, urinary tract infections, vaginal injuries, dyspareunia, urine retention, and mesh erosion are the common complications in sling procedures. The most frequent and important complications of the retropubic mid-urethral sling placement procedure are bladder perforation, voiding dysfunction, and the development of urinary urgency symptoms. Similar to retropubic slings, TOT slings may result in voiding dysfunction [27, 28]. The reason for less frequent voiding disorders in the TOT procedure is that the tape passes horizontally through the suburethra and thus makes no kink. Additionally, the major complication rate is quite low compared to other sling procedures since the surgical maneuvers in the TOT procedure are far from the retropubic region [18, 20]. While no major complications were observed in any of the participants in our study, the most common complication observed was groin pain which was resolved within the first 2 weeks.

The limitations of the current study were that there were no comparisons with other sling procedures, and there was a lack of long-term follow-up results. The strengths of the present study include the sufficient number of participants and the representative sample from central Turkey. Therefore, the results of this study could be generalized to the majority of the country. Another strength of our study is that it was a prospective investigation, and the same senior surgeons performed the TOT procedures.

Conclusion

In conclusion, this study demonstrated that the TOT procedure is an easy-to-apply, minimally invasive technique with low peri- and postoperative complication rates and a high success rate in appropriate and correct indications. Additionally, the TOT procedure improves the quality of life of the patient and reduces morbidity. Nevertheless, further studies with larger cohorts are needed to validate the results of the current study and determine the long-term effects.

Conflict of Interest

The authors declare no conflict of interest.

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Characteristics of atherosclerotic plaque and the thickness of the carotid artery intima-media complex in patients with rheumatoid arthritis

Karakteristike aterosklerotskog plaka i debljina intima-medija kompleksa karotidnih arterija kod bolesnika sa reumatoidnim artritisom

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Abstract

Background/Aim. Rheumatoid arthritis (RA) represents an independent risk factor for the development of cardiovascular (CV) disease (CVD). Early detection of atherosclerotic changes is of tremendous importance in the prevention of CV events. An increase in the carotid artery intima-media thickness (cIMT) is considered a sensitive marker of early subclinical atherosclerosis. The aim of our investigation was to assess the cIMT, the number and type of carotid plaques (CPs), and the severity of carotid artery stenosis in RA patients. Furthermore, we investigated the correlation between all the above-mentioned parameters and disease duration and activity. **Methods**. The research included 92 participants, of which 58 were patients with RA, and the remaining 34 participants were healthy individuals (control group). In patients with RA, clinical examination and laboratory findings were used for assessing disease activity. All participants underwent a color Doppler ultrasound examination of the carotid arteries with a linear probe in order to assess cIMT, the number and type of CPs, as well as the severity of stenotic lesions. **Results.** The mean cIMT in RA patients was statistically significantly higher compared to the control group (0.8 ± 0.2 mm vs. 0.7 ± 0.2 mm; $p < 0.01$). CPs were found in 34 out of 58 RA patients (58.6%) and 4 out of 34 (11.8%) participants in the control group ($p < 0.001$). The number of CPs per patient was significantly higher in the RA group compared to the control group (1.4 ± 0.9 vs. 0.2 ± 0.4; $p < 0.001$). The cIMT, the presence and number of CPs, and the severity of carotid artery stenosis were not statistically significantly related to disease activity. There was a statistically significant direct correlation between the duration of RA and the percentage of carotid arterial stenosis ($r = 0.320$, $p = 0.034$). **Conclusion.** The cIMT and the presence and number of CPs per patient were significantly higher in RA patients. Moreover, there was a positive correlation between RA disease duration and the severity of carotid artery stenosis. This study showed that RA represents an independent risk factor for an increase in cIMT and the development of subclinical atherosclerosis.

Key words:
arthritis, rheumatoid; atherosclerosis; cardiovascular disease; carotid arteries; plaque, atherosclerotic; risk factors.

Apstrakt

Uvod/Cilj. Reumatoidni artritis (RA) predstavlja nezavisni faktor rizika od nastanka kardiovaskularnih (KV) oboljenja (KVO). Rano otkrivanje aterosklerotskih promena je ključno u prevenciji KV događaja. Povećanje debljine kompleksa intima-medija (DKIM) na karotidnim arterijama smatra se senzitivnim markerom rane, subkliničke ateroskleroze. Cilj našeg ispitivanja bio je da procenimo DKIM, broj i tip karotidnih plakova (KP), težinu stenoze karotidnih arterija kod bolesnika sa RA kao i povezanost navedenih parametara sa dužinom trajanja i aktivnosti bolesti. **Metode**. U studiju je bilo uključeno 92 učesnika, od kojih je njih 58 imalo RA, a preostalih 34 bile su zdrave osobe (kontrolna grupa). Kod bolesnika sa RA procena aktivnosti bolesti vršena je na osnovu kliničkog pregleda i laboratorijskih analiza. Svim ispitanicima urađen je kolor Dopler ultrazvučni pregled karotidnih arterija kojim su procenjivani DKIM, broj i tip KP, kao i težina stenoznih lezija. **Rezultati.** Bolesnici sa RA imali su statistički značajno veće vrednosti DKIM u odnosu na kontrolnu grupu (0,8 ± 0,2 mm vs. 0,7 ± 0,2 mm; $p < 0,01$). KP registrovani su kod 34 od ukupno 58 bolesnika iz RA grupe (58,6%) i kod 4 od ukupno 34 (11,8%) osoba iz kontrolne grupe ($p < 0,001$). Broj KP po ispitaniku bio je statistički značajno veći kod bolesnika sa RA u odnosu na

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kontrolnu grupu (1,4 ± 0,9 vs. 0,2 ± 0,4; $p < 0.001$). DKIM, prisustvo i broj KP kao i težina stenoza karotidnih arterija nisu se statistički razlikovali u odnosu na aktivnost bolesti. Postojala je statistički značajna korelacija dužine trajanja RA i procenata stenozirajućih promena ($r = 0,320$, $p = 0,034$) **Zaključak.** Kod bolesnika sa RA detektovana je značajno veća vrednost DKIM, kao i veća zastupljenost i broj KP po ispitaniku. Postojala je pozitivna korelacija između dužine trajanja RA i težine stenozirajućih lezija. Ova studija pokazala je da RA predstavlja nezavisni faktor rizika za povećanje DKIM i nastanak subkliničke ateroskleroze.

Ključne reči:
artritis, reumatoidni; ateroskleroza; kardiovaskularne bolesti; aa. carotis; aterosklerotički plak; faktori rizika.

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by erosive synovitis, and, despite therapy, it often leads to progressive joint damage [1]. In addition, an increased incidence of cardiovascular (CV) disease (CVD) has been identified, and this cannot be explained by traditional risk factors. It has been proven that RA poses an independent risk for the development of CVD [2].

In RA patients, all stages of the atherogenic process are accelerated. Systemic inflammation underlying RA is an independent risk factor for CVD [3]. The primary inflammation site is the synovial tissue which releases pleiotropic cytokines, such as TNF-α, IL-1, and IL-6 [4]. They perform the role of mediator in numerous metabolic processes – through effects on the liver, skeletal muscles, adipose tissue, and endothelium. That generates proatherogenic changes, prothrombotic effects, and endothelial dysfunction [5]. In patients with a high risk of CVD, early detection of atherosclerotic changes is of tremendous importance in the prevention of CV events that may cause irreversible damage [6]. Today, an increase in the thickness of the carotid artery intima-media complex (cIMT) is considered a sensitive marker of early subclinical atherosclerosis [7].

The aim of our paper was to assess the cIMT, the number and type of carotid plaques (CPs), and the percentage of carotid artery stenosis in RA patients. Furthermore, we investigated the correlation between all the above-mentioned parameters and disease duration and activity.

Methods

Recruitment and patients

The research included 92 participants, of which 58 were RA patients (RA group), and 34 were healthy individuals (control group). All participants in the RA group fulfilled the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for the diagnosis of RA [8]. Likewise, all RA patients were receiving stable treatment with the disease-modifying drug Methotrexate (MTX), 12.5 to 17.5 mg weekly.

All RA patients were diagnosed with RA at least six months before inclusion into the study. Additionally, all RA participants were on stable therapy with MTX for at least three months prior to the beginning of the study.

Patients receiving corticosteroid, biological, or statin therapy were not included in the study. Likewise, patients who received intra-articular corticosteroids in the last three months were not included in the study. Furthermore, patients who had survived a stroke, transient ischaemic attack, or patients suffering from peripheral arterial disease or proven coronary artery disease (patients who survived a heart attack or had any type of myocardial revascularization) were not included in the study. Further exclusion criteria for participation in the study were the existence of systemic autoimmune disease (i.e., systemic lupus erythematosus, Crohn's disease) or malignancy.

The study was approved by the Ethics Committee of the Institute for treatment and rehabilitation Niška Banja, Serbia (No 03-8382/1 from 27 March, 2019).

Study design

All patients underwent a clinical examination (tender joints and/or swelling) and laboratory tests: sedimentation (SE), C-reactive protein (CRP), and rheumatoid factor (RF). In patients with RA, clinical examination and laboratory findings were used for assessing (calculating) disease activity. The disease activity is presented via Disease Activity Score-28 (DAS28) based on erythrocyte sedimentation rate and Clinical Disease Activity Index (CDAI) [9, 10].

All participants underwent color Doppler ultrasonography of the carotid arteries with a linear probe, and they had the cIMT, type and number of CPs, and the percentage of carotid artery stenosis estimated.

Color Doppler ultrasonography

Color Doppler ultrasound examination of the carotid arteries was done in all the examinees using the ESAOTE My Lab60 Xvision, with a 4–13 MHz multi-frequency linear probe machine, assessing cIMT, number and type of CPs, and percentage of carotid artery stenosis. Intraluminal lesions were documented using B-mode imaging. cIMT was measured in the posterior wall of the common carotid artery, 2 cm away from the bifurcation apex, in the region without focal changes. On the other hand, for the analysis of stenotic lesions and plaque properties, we used bilateral longitudinal images of the internal, external, and common carotid arteries.

A carotid artery plaque was defined as a localized protrusion of the vessel wall, which extended into the lumen ≥ 1.5 mm or had a thickness exceeding the intima-media thickness of the adjacent portion of the vessel wall by > 50% according to Mannheim Intima-Media Thickness Consensus [11]. The plaque morphology was defined in terms

of its echogenicity as lipid, fibrolipid, fibrous, fibrocalcific, and calcified [12]. The percentage of stenosis was determined according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) guidelines [13]. Carotid stenoses were described in accordance with the Consensus Panel Gray-Scale and Doppler Ultrasound Criteria for Diagnosis reported by Grant et al. [14], where the degree of stenosis was classified into the categories of normal (no stenosis), < 50% of stenosis, 50%–69% of stenosis, > 70% of stenosis to near occlusion, near occlusion, and total occlusion, according to Piepoli et al. [15].

Statistical analysis

The data are presented in the form of descriptive measures – arithmetic means, standard deviations, and absolute and relative frequencies. The normality of data was tested by the Kolmogorov-Smirnov test. Due to significant deviation from a normal distribution, measures of central tendencies of the subsamples were compared by the Kruskal-Wallis test, while the Mann-Whitney test was used in a post-hoc analysis. Frequencies of categorical data of the subsamples were compared by the Chi-squared test. In all tests, the level of significance was set to $\alpha = 0.05$. The regression analysis was used to determine the relationship between disease duration and cIMT and plaque characteristics. All statistical procedures were carried out using the R package.

Results

The research was conducted on 58 RA patients (24 men and 34 women) with an average age of 59.8 ± 9.9 years and an average disease duration of 8.3 ± 6.2 years. The control group consisted of 34 healthy participants with an average age of 60.9 ± 0.9 years.

There was no significant difference in the presence of risk factors for the development of atherosclerosis between the groups (Table 1).

The mean cIMT in RA patients was 0.8 ± 0.2 mm, which was statistically significantly higher ($p < 0.01$) compared to the control group, where the cIMT was 0.7 ± 0.2 mm. Atherosclerotic CPs were found in 34 out of 58 RA patients (58.6%) and 4 out of 34 (11.8%) examinees in the control group ($p < 0.001$). The number of CPs per patient in the RA group was 1.4 ± 0.9, which was statistically significantly higher ($p < 0.001$) compared to the control group, where the number of CPs was 0.2 ± 0.4. The percentage of carotid artery stenosis (26.4 ± 14.3% vs. 3.7 ± 8.3%) was also statistically significantly higher in the RA group ($p < 0.001$) (Table 2).

Nineteen patients in the RA group (32.8%) had cIMT ≥ 0.9 mm, which is considered a pathological finding. On the other hand, only one participant (2.9%) in the control group had cIMT ≥ 0.9 mm. Type 4 CP (fibrocalcific) was predominant in 13 out of 34 examinees (38.2%), followed by type 5 CP (calcified) in 11 out of 34 (32.4%) examinees, while type 3 CP (fibrous) was the least represented with 10 out of 34 (29.4%) participants. None of the participants had type 1 CP (lipid) or type 2 CP (fibrolipid).

Disease activity in RA patients was determined based on clinical examination and laboratory analyses using DAS28 and CDAI scores. All patients were divided into 3 groups according to the disease activity rate expressed via DAS28. The first group (high disease activity), with DAS28 > 5.1, encompassed 21 out of 58 patients (36.2%); the second group (moderate disease activity), with DAS28 ≥ 3.2 and ≤ 5.1, included 22 out of 58 (37.9%) patients; the third group (low disease activity and remission), with DAS28 < 3.2, consisted of 15 out of 58 patients (25.9%).

Concerning the CDAI disease activity index, high disease activity (CDAI: 22.1–76) was present in 16 out of 58 (27.6%) patients, while the moderate activity group (CDAI: 10.1–22.0) included 28 out of 58 (48.3%) patients, and the low disease activity and remission group (CDAI: 0.0–10.0) encompassed 14 out of 58 (24.1%) patients.

Table 1

Comparison of cardiovascular risk factors between participants with rheumatoid arthritis (RA) and without RA

Risk factors	RA group	Control group	*p*-value
Arterial hypertension, n (%)	33 (56.9)	16 (47.1)	0.473
Hyperlipidemia, n (%)	15 (25.9)	8 (23.5)	0.955
Smoking, n (%)	16 (27.6)	9 (26.5)	0.947
Diabetes mellitus, n (%)	3 (5.2)	0 (0)	0.757
Body mass index, mean ± SD	26.5 ± 4.5	26.4 ± 4.6	0.928

SD – standard deviation.

Table 2

Comparison of carotid artery intima-media thickness (cIMT) and plaque characteristics in participants with rheumatoid arthritis (RA) and without RA

Carotid artery characteristics	RA group	Control group	*p*-value
cIMT (mm), mean ± SD	0.8 ± 0.2	0.7 ± 0.2	< 0.01
CPs, n (%)	34 (58.6)	4 (11.8)	< 0.001
Number of CPs, mean ± SD	1.4 ± 0.9	0.2 ± 0.4	< 0.001
% stenosis, mean ± SD	26.4 ± 14.3	3.7 ± 8.3	< 0.001

SD – standard deviation; CPs – carotid plaques.

Finally, we decided to compare the parameters obtained by Doppler ultrasound of the blood vessels of the neck with the parameters of disease activity in the group of RA patients. The results of our research showed that the cIMT, the presence and number of CPs per patient, and the percentage of carotid artery stenosis were not statistically significantly related to disease activity expressed through DAS28 and CDAI scores (Tables 3 and 4).

The cIMT was significantly higher in patients with a positive RF factor ($p < 0.05$), while the other parameters tested (the percentage of stenosis and the presence and number of CPs) were not associated with RF positivity.

The correlation analysis demonstrated that there is a statistically significant direct correlation between the duration of RA and the percentage of carotid arterial stenosis ($\rho = 0.320$, $p = 0.034$); however, no statistically significant difference was found between the duration of RA and other examined parameters (cIMT and the presence and number of CPs per patient) (Figure 1).

Table 3

Characteristics of carotid arteries in relation to disease activity expressed via Disease Activity Score-28 (DAS28)

Parameter	DAS28			*p*-value
	> 5.1	≥ 3.2–≤ 5.1	< 3.2	
cIMT (mm), mean ± SD	0.8 ± 0.2	0.3 ± 0.2	0.8 ± 0.2	0.815
CPs, n (%)	13 (61.9)	13 (59.1)	8 (53.3)	0.738
Number of CPs, mean ± SD	1.5 ± 1.25	1.4 ± 1.1	1.25 ± 1.4	0.715
% stenosis, mean ± SD	27.3 ± 19.9	26.2 ± 21.9	24.0 ± 22.1	0.165

cIMT – carotid artery intima-media thickness; SD – standard deviation; CPs – carotid plaques.

Table 4

Characteristics of carotid arteries in relation to disease activity expressed via clinical disease activity index (CDAI)

Parameter	CDAI			*p*-value
	22.1–76.0	10.1–22.0	0.0–10.0	
cIMT (mm), mean ± SD	0.8 ± 0.2	0.8 ± 0.3	0.8 ± 0.2	0.825
CPs, n (%)	10 (62.5)	16 (57.1)	8 (57.1)	0.846
Number of CPs, mean ± SD	1.50 ± 1.47	1.4 ± 1.2	1.4 ± 1.4	0.738
% stenosis, mean ± SD	27.3 ± 24.8	28.1 ± 26.1	22.6 ± 21.64	0.175

cIMT – carotid artery intima-media thickness; SD – standard deviation; CPs – carotid plaques.

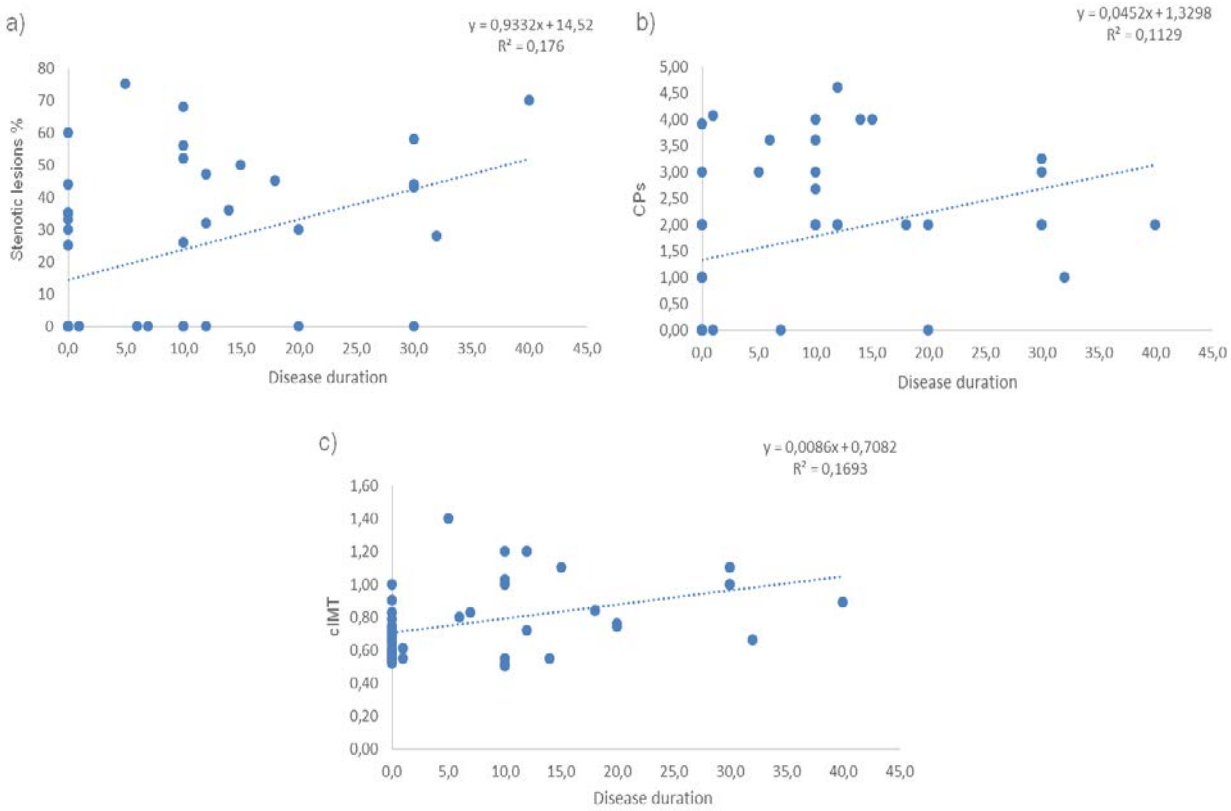

Fig. 1 – Correlation between disease duration and: (a) stenotic lesions %; (b) number of carotid plaques (CPs); (c) the carotid artery intima-media thickness (cIMT).

Discussion

The true frequency of CVD in RA patients is difficult to estimate accurately because they tend to remain asymptomatic [16]. Moreover, if symptoms do occur, they are usually atypical. That is why patients with RA are more likely to experience unrecognized myocardial infarction or death as a result of sudden cardiac failure [17]. On the other hand, it is well-known that patients with RA have a 1.5–2-fold higher incidence of CVD than the general population [16, 18, 19]. Furthermore, life expectancy in RA patients is shortened to six, seven years [20], mainly due to higher CV mortality [21].

The pathogenesis of CVD in RA is rather complex and includes chronic inflammation and immune dysregulation [1]. Both disorders cause endothelial and cardiomyocyte dysfunction [16], which leads to atherosclerosis and/or (ischemic or non-ischemic) heart failure [22]. Atherosclerosis, as a chronic, systemic, and inflammatory disease, can affect any artery in the human body. However, it usually causes the narrowing of coronary and cerebrovascular arteries, which can lead to life-threatening events like acute coronary syndrome (ACS) or stroke. Mainly due to these two disorders, CVDs are the leading cause of death worldwide, with 17.8 million deaths per year [23]. That is why early detection of atherosclerotic changes is of tremendous importance.

The presence of atherosclerotic CP has proved to strictly correlate with atherosclerotic changes in coronary arteries, while an increase in cIMT correlates with manifested coronary artery disease [14, 24]. Namely, a cIMT analysis in a large population study conducted on over 15,000 people in the USA showed that an increase in cIMT by 0.2 mm leads to an increase of the relative risk of myocardial infarction by 33% and cerebral infarction by 28% [25]. Another group of authors showed that the increase in cIMT by 0.2 mm even doubles the risk of CV events [26]. Likewise, an increase in the cIMT is considered a sensitive marker of early subclinical atherosclerosis [27]. Some authors suggest using von Willebrand factor (vWF) activity as an early marker of subclinical atherosclerosis in RA [28]. That being the case, we chose to do a carotid artery examination with high-resolution ultrasound. The ultrasound examination of carotid arteries represents a reliable, accurate, and easily accessible technique that detects atherosclerosis in subclinical stages [27].

In our study, patients with RA had higher values of cIMT and a greater number of CPs compared to the control group. That is not a "breakthrough finding", as patients with RA usually have higher values of IMT and plaques compared to the healthy population [27, 29]. That refers not only to the carotid [30] but also to the femoral arteries [31]. In a meta-analysis by van Sijl et al. [32], which included 22 studies that investigated values of cIMT in RA patients only in one study, cIMT was smaller than in the healthy population. All other studies showed higher cIMT values in RA patients [29]. However, this meta-analysis had some flaws because, in most studies, patients with RA had more CV risk factors compared to the healthy control. In addition, there was a variation in ultrasound protocols among studies used for cIMT estimation [32]. Mohan et al. [33] also showed that RA patients have higher values of cIMT compared to healthy populations, stressing the point that this finding "appears to be a useful surrogate marker for detecting subclinical atherosclerosis in adults". Saigel et al. [34] went even further by suggesting routine screening for silent atherosclerosis in all RA patients by ultrasound examination of cIMT. Ristić et al. [29] showed that RA represents an independent risk factor for atherosclerosis and, what is even more important, long-term anti-inflammatory therapy may decrease cIMT.

CPs are also commonly found in patients with RA [2]. In fact, patients with RA have a 3-fold higher risk for CP development [35]. In a study by Mahajan et al. [36], the difference in the prevalence of CP between RA and healthy subjects was even more pronounced, as 21% of RA patients and only 1% of control group participants had CP. Once again, our study showed that RA patients have significantly higher values of cIMT and a greater number of CPs compared to the healthy population. That is of great importance as both parameters, cIMT and CP, carry high predictive power for the development of CV events [37].

Given that the higher incidence of CVD in RA patients is explained by systemic inflammation, a positive correlation between RA activity and the severity of atherosclerotic carotid lesions is expected. However, the results obtained in larger studies are contradictory. Patel et al. [38] have demonstrated the existence of a correlation between the cIMT and disease activity in RA patients, who were divided into low, moderate, and high disease activity groups. Likewise, in 2019, Abd El-Monem et al. [39] showed a positive correlation between cIMT and disease activity expressed in DAS28. Furthermore, compared to RA patients in remission, RA patients with active disease seem to have less stable and more vulnerable plaques, which increases the probability of an acute CV event [40]. RA activity represents an independent risk factor for impaired glucose metabolism, known to be an independent risk factor for CVD [41]. In contrast, Jonsson et al. [42], Roman et al. [35], and Cuomo et al. [43] have shown in their research that there is no correlation between cIMT and disease activity, while Semb et al. [40] stated that there is a positive correlation between CP vulnerability and RA disease activity assessed with CDAI but not with DAS28. In our research, disease activity was expressed through DAS28 and CDAI indices. cIMT, the number of CPs per patient, and the percentage of carotid arterial stenosis were the lowest in the group of patients who were in remission or had low disease activity. On the other hand, our study didn't find a significant correlation in the severity of atherosclerotic lesions with the group of moderate and high disease activity. Similar findings were observed in an Italian study with young RA patients [44]. In fact, even patients with continued low RA disease activity appear to suffer from atherosclerotic lesions [44, 45]. However, this does not diminish the need for aggressive control of RA activity because chronic inflammation is probably the driving force for premature atherosclerosis.

In our study, there was a positive correlation between the duration of RA and the percentage of stenotic lesions; however, there was no correlation between the duration of RA and other parameters tested (cIMT, number of CPs per

patient, and type of plaque). These findings are in contrast with previous studies [35, 46, 47]. There are two possible explanations for why such results were obtained. First is the duration of RA. Namely, in most conducted studies, RA lasted for more than 10 years [39], and in our study, the average duration of the disease was about 8 years. However, in the Del Rincon et al. [46] study, the borderline for higher values of cIMT was lower – seven years [46]. Many studies support the assumption that disease duration has more impact on the development of atherosclerosis than the disease activity [48, 49]. However, our study failed to confirm this hypothesis. That highlights the need for more prospective studies on a larger number of patients, which will confirm or reject this allegation. The second explanation is that the obtained results are influenced by the therapeutic approach. For instance, the positive correlation between the cIMT and disease duration in the study by Kumeda et al. [31] can be explained by the small number of patients receiving MTX (12%). In studies where RA patients were intensively treated with MTX (54%–98%), the correlation between the cIMT and disease duration was not significant [30, 50]. In our study, there was no correlation between disease duration and cIMT, probably due to the early aggressive treatment and the fact that all patients were on MTX therapy.

Finally, many authors [49, 51] suggest that the existence of the RF factor (RF positivity) is a risk factor for the development of atherosclerosis [52]. Moreover, RA patients with RF seropositivity have a higher incidence of ACS and a worse prognosis after acute myocardial infarction [53]. That is why the European Association of Rheumatologists defines seropositivity (alongside high disease activity and RA duration over 10 years) as the main CV risk factor [54]. In our study, cIMT was significantly greater in patients with a positive RF factor, while other parameters of the carotid arteries tested were not associated with RF positivity.

Conclusion

The results of our study showed that the cIMT, the presence and number of CPs per patient, and the percentage of carotid artery stenosis were statistically significantly higher in RA patients. Moreover, there was a positive correlation between disease duration and the severity of carotid stenotic lesions. Our study showed that RA represents an independent risk factor for an increase in cIMT and the development of subclinical atherosclerosis.

Conflict of interest

The authors declare no conflict of interest regarding the present paper.

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CASE REPORTS

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Successful hysteroscopic management of two cases of interstitial pregnancy

Uspešno histeroskopsko rešavanje dva slučaja intersticijalne trudnoće

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Abstract

Introduction. Interstitial pregnancy (IP) is the rarest type of tubal pregnancy with a high rupture rate and often remains asymptomatic in the first 10–12 gestational weeks. Therefore, the timing of the diagnosis is crucial for successful management. **Case report.** Two patients, aged 28 and 22, were diagnosed with IP using transvaginal ultrasound. Both patients were asymptomatic, with initial serum βhCG of 6,664 mIU/mL and 4,641 mIU/mL, respectively. Since they refused treatment with methotrexate and wanted to preserve their fertility, we performed operative hysteroscopy with resection and evacuation of the gestational tissue. The procedures were uneventful. The βhCG levels dropped significantly, and the patients were discharged after three and four hospital days, respectively. **Conclusion.** Using hysteroscopic procedures, we successfully treated two asymptomatic patients with IP of gestational age < 10 weeks by ultrasonography and levels of serum βhCG < 7,000 mIU/mL. With the occurrence of IP but also the numerous advantages of hysteroscopy, large, multicenter studies are necessary to further investigate the place of this approach as a single treatment method for IP. Trends and consequences observed during the COVID-19 pandemic correlate with the importance of timely diagnosis of ectopic pregnancies, the benefits of a minimally invasive approach in their treatment, and epidemiologically justified shorter hospital stays.

Key words:
gynecologic surgical procedures; minimally invasive surgical procedures; pregnancy, ectopic.

Apstrakt

Uvod. Intersticijalna trudnoća (IT) je najređa forma tubarne trudnoće koja ima visoku stopu rupture i često ostaje asimptomatska u prvih 10–12 nedelja gestacije. Stoga, vreme kada se postavi dijagnoza je ključno za uspešno lečenje. **Prikaz bolesnika.** Kod dve pacijentkinje, starosti 28 i 22 godine, ultrazvučno je dijagnostikovana IT. Obe pacijentkinje bile su bez simptoma, a koncentracije njihovog serumskog βhCG-a iznosile su 6 664 odnosno 4 641 mIU/mL. S obzirom na to da su odbile lečenje metotreksatom i imale želju da sačuvaju svoju fertilnost, podvrgnute su histeroskopskoj resekciji i evakuaciji gestacijskog tkiva. Operativne procedure su prošle bez komplikacija. Nakon operacija, značajno su snižene koncentracije βhCG-a u serumu i pacijentkinje su otpuštene trećeg, odnosno četvrtog postoperativnog dana. **Zaključak.** Primenom histeroskopske resekcije uspešno smo rešili IT kod dve pacijentkinje bez simptoma, sa IT gestacijske starosti kraće od 10 nedelja i nivoima serumskih βhCG-a nižim od 7 000 mIU/mL. S obzirom na izuzetno retku pojavu IT, ali i mnogobrojne prednosti histeroskopije, neophodne su obimne, multicentrične studije, da bi se ispitali mesto i značaj tog pristupa u rešavanju IT. Obrasci i posledice uočeni tokom COVID-19 pandemije su u korelaciji sa značajem pravovremene dijagnostike ektopičnih trudnoća, minimalno invazivnim pristupom u njihovom rešavanju i epidemiološki opravdanom kraćom hospitalizacijom.

Ključne reči:
hirurgija, ginekološka, procedure; hirurgija, minimalno invazivne procedure; trudnoća, ektopična.

Introduction

Despite the ongoing confusion regarding the terminology used by practitioners and scholars, interstitial, angular, and cornual pregnancies represent the specific types of pregnancies with contrasting prognoses and management routes.

According to the literature, interstitial pregnancy (IP) is the rarest type of tubal pregnancy and accounts for about 2–4% of all ectopic pregnancies (EPs) [1]. Regardless of its incidence, IPs account for about 20% of deaths related to EPs [1, 2]. The presence of the gestational sac in the proximal part of the fallopian tube surrounded by a thin layer of muscular fibers of the uterus reportedly has a rupture rate of almost 14% [1, 2]. These reported percentages, and the fact that most IPs remain asymptomatic until 7 to 12 weeks of gestation, urge for the correct and prompt diagnosis and an adequate method of clinical management.

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Many factors determine the adequate treatment of IP. Of these, the most important is the presence of life-threatening signs and symptoms. Therefore, the timing of the diagnosis is crucial. The patient's age and fertility should always be considered in choosing the best treatment option. Nowadays, the combination of serial measurements of serum human beta chorionic gonadotropin (βhCG) and three-dimensional transvaginal ultrasound significantly shorten the time needed for the correct diagnosis and provide a more conservative approach, either medical or surgical [3]. In the era of modern gynecology, endoscopy is considered a golden standard for the treatment of EPs in unusual locations [4]. Literature reports laparoscopy, hysteroscopy, and methotrexate (MTX) as single or combined options in treating IPs.

We present two cases of IPs successfully managed solely by operative hysteroscopy.

Case report

Case I

A 28-year-old patient, para 4, gravida 5, was admitted to the Clinic under the ultrasonographic suspicion of EP. The period of amenorrhoea was eight weeks. The initial value of serum βhCG was 6,664 mIU/mL [reference range (RR) < 5.00 mIU/mL; for gestational weeks 1–10, RR is 202.00 – 231,000.00 mIU/mL]. An ultrasonographic exam revealed a gestational sac of 20 mm in diameter in the right uterine *cornua* with the interstitial sign (Figure 1A). There were no signs of intrauterine pregnancy, and both adnexa appeared morphologically normal. The pouch of Douglas and the vesicouterine space were free of any fluid. The patient showed no symptoms of pain, bleeding, or weakness. A laboratory exam did not show any signs of blood loss. Considering the patient's age and future fertility, along with the ultrasonographic and clinical features, we performed diagnostical hysteroscopy. Under general anesthesia, a 10 mm operative hysteroscopy revealed an empty uterine cavity, with the decidual transformation of the endometrium and a gestational sac in the region of the right uterine *cornua* (Figure 1B). The gestational sac was resected and then evacuated from the uterine cavity using a 5Fr hysteroscopic resectoscope. A bipolar electrode was used for the hemostasis. The blood loss was insignificant. Levels of βhCG significantly dropped two days after the procedure (611 mIU/mL), and the patient was discharged.

Case II

A 22-year-old patient, para 0, gravida 0, was referred from the primary healthcare unit with the diagnosis of a missed abortion. The period of amenorrhoea was eleven weeks. Serum βhCG was 4,641 mIU/mL (for gestational weeks 11–15, RR is 22,536.00–234,990.00 mIU/mL). The patient did not complain of any symptoms. At the ultrasonographic exam, there were no signs of intrauterine pregnancy. A gestational sac of 47 mm, with an embryo whose crown-lump length was 3.5 mm, without cardiac activity, was seen in the right uterine *cornua* (Figure 2A). Bearing in

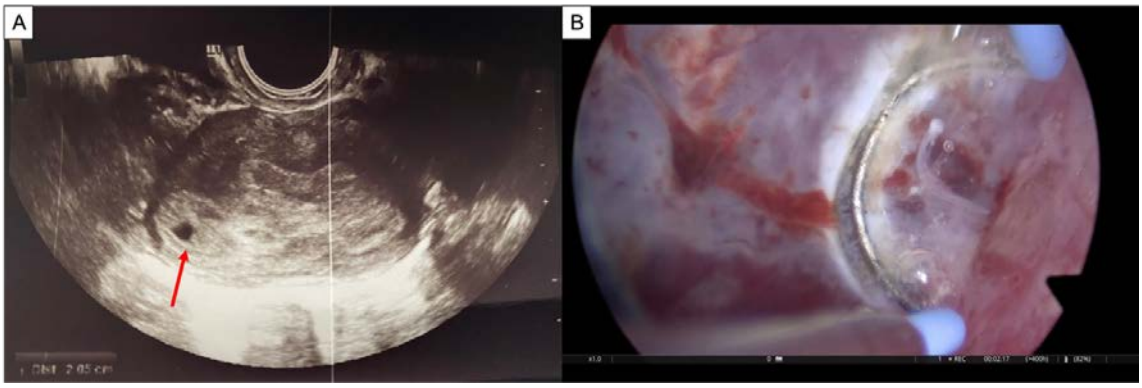

Fig. 1 – A) Ultrasonographic feature from Case I: a gestational sac of 20 mm (red arrow) in the right uterine *cornua* with the interstitial sign; B) View of the gestational sac at the moment of hysteroscopic resection.

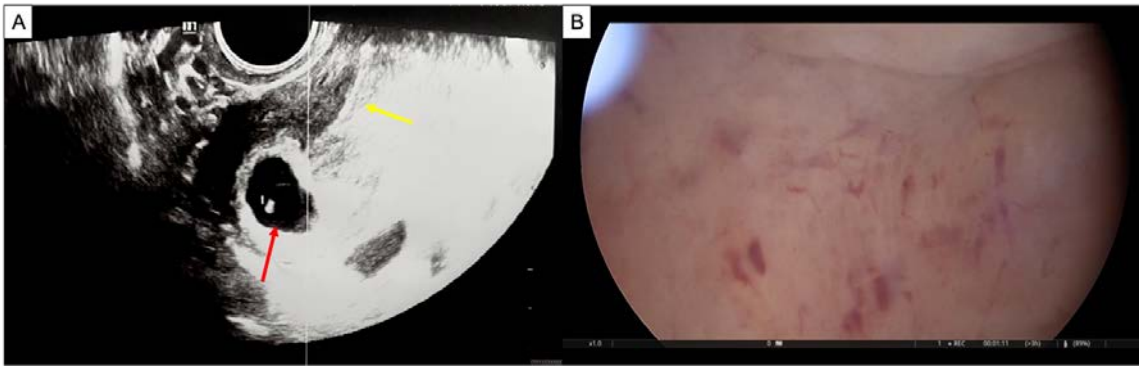

Fig. 2 – A) Ultrasonographic feature from Case II: gestational sac of 47 mm (red arrow), with an embryo, crown-lump length of 3.5 mm, without cardiac activity, in the right uterine *cornua* alongside an empty uterine cavity (yellow arrow); B) Hysteroscopic view of an empty uterine cavity.

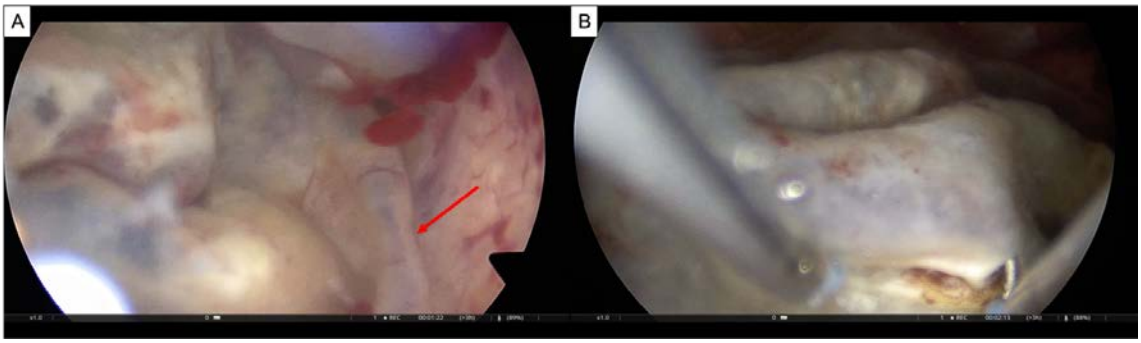

Fig. 3 – A) Hysteroscopic view of distended right uterine *cornua* with the collapsed gestational tissue in the second patient; B) Hysteroscopic resection of the gestational tissue.

Table 1

Patients' obstetric history, onset features, duration of the procedures, and hemoglobin (Hgb) levels

Pts	Age (years)	Obstetric history	Serum βhCG (mIU/mL)	Onset symptoms	Ultrasonographic feature(s)	Duration of the procedure (min)	[1] Hgb before the procedure (g/L)	Hgb after the procedure (g/L)	Hospital days
I	28	P4G5	6,664	none	A gestational sac of 20 mm in diameter in the right uterine *cornua*, with the interstitial sign.	13	150	124	4
II	22	P0G0	4,641	none	A gestational sac of 47 mm in the right uterine *cornua*, with an embryo whose CRL was 3.5 mm, without cardiac activity.	27	141	122	3

[1] Note: reference range for hemoglobin (Hgb) is 119–157 g/L.

Pts – patients; βhCG – beta chorionic gonadotropin; CRL – crown-lump length.

mind the patient's obstetrical history and future fertility, a diagnostic hysteroscopy was arranged. Hysteroscopy revealed an empty uterine cavity (Figure 2B) and distended right uterine *cornua* with collapsed gestational tissue (Figure 3A). Using the same approach described in the previous patient, gestational tissue was resected (Figure 3B) and evacuated from the uterine *cornua*. The blood loss during the procedure was negligible. Serum βhCG level saw a significant drop following the procedure, and the patient was discharged on the second postoperative day.

Obstetrical history, ultrasonographic findings, initial serum βhCG concentrations, number of hospital days, and levels of hemoglobin (Hgb) before and after the procedure for both patients are presented in Table 1.

Discussion

As previously mentioned, distinguishing the IP and eccentric intrauterine pregnancies (cornual and angular pregnancies) is essential. Angular pregnancy, the implantation of the gestational sac in the lateral angle of the uterine cavity, medially to the uterotubal junction, is a form of the eccentric intrauterine pregnancy with a live birth rate of 25–80% [1, 5]. Cornual pregnancy represents the presence of the gestational sac in the rudimentary horn of the uterus with the Mullerian anomaly. There are several proposed ultrasonographic criteria for the correct diagnosis of IP: empty uterine cavity, gestational sac separated from the lateral edge of the cavity and surrounded by a thin myometrial layer (introduced in the 1990s by Timor-Tritsch et al. [6]), and interstitial line sign [7]. Combined with the double sac sign, these criteria can distinguish IP and eccentric pregnancies with a specificity of 100% [1]. The importance of distinguishing between these entities lies in the fact that IP has a two-fold higher mortality rate compared to other EPs [3].

There are few reported methods for the successful treatment of IP. The most conservative, expectant method, is not recommended because of the known, life-threatening complications of the IP.

Medical treatment with systemic or local injection of MTX is widely described as an optimal method for patients who wish to preserve their fertility. Nevertheless, this treatment option has a failure rate of 9–65% [8]. Moreover, systemic administration of MTX could cause serious side effects. Additionally, this conservative medical option should be used with caution in cases when βhCG > 5,000 mIU/mL and

the identified gestational sac is > 5cm [8]. Ultrasonography-guided local instillation of the potassium chloride was recently described as a novel alternative after unsuccessful MTX treatment of IP [9].

Since the operator can see and treat the pathology found during the procedure, hysteroscopy is considered a golden standard in managing intrauterine pathology. Compared to MTX, hysteroscopy requires less time for the normalization of βhCG levels and shorter hospital stays in EP treatment [10]. One of the first described cases of hysteroscopic management of IP was published by Meyer and Mithcell [11]. Following this report, hysteroscopy was often performed combined with laparoscopy for managing IP. In a systematic literature review, D'hoore et al. reported 8 articles describing the hysteroscopic treatment of IP or interstitially located retained products of conception, including their case report [12]. None of the published cases or case series used hysteroscopic resection and evacuation of the gestational tissue as a single treatment option. We have identified nine additional published papers regarding the management of IP. Besides four papers regarding the laparoscopic approach, one laparotomy, and one MTX treatment, one group of authors reported successfully combined laparoscopic and hysteroscopic treatment of IP. In a case series of laparoscopic-assisted hysteroscopic removal of IP, Niu et al. [13] concluded that if the mass distends the uterine *cornua* with a tunnel connecting the mass and the *cornua*, hysteroscopic resection is likely to be successful. In a series of five patients, Casadio et al. [14] successfully treated IP with local hysteroscopic MTX injection. Hysteroscopic-assisted local MTX injection could minimize adverse systemic effects of MTX [14, 15]. This modality, combined with our report of two cases, seems promising for managing IP. Hysteroscopy, with or without local MTX injection, should be a subject for future studies to determine whether it could be used without the need for laparoscopic assistance.

Furthermore, detailed investigations are necessary to identify the patients most suitable for this modality. With the precise identification of suitable patients, the main advantages of hysteroscopic treatment alone could be seen in the short duration of the procedure, fewer hospital days, and the ability for the patients to conceive shortly after the next menstrual cycle.

The main limitation of our study is the small number of subjects. Even though we successfully treated two patients with IP using operative hysteroscopy, future multicenter-based studies with a significantly larger number of women with IP treated solely with this procedure are needed. Moreover, along with more subjects, the potential benefits of hysteroscopy compared to medical or invasive surgical treatment of IP could be observed in well-designed comparative studies. On the other hand, the occurrence of IP presents the main obstacle to overcoming the mentioned limitations.

Additionally, we have observed another potential advantage of the endoscopic treatment of EPs, along with the IPs. Several authors have pointed out the higher rates of the ruptures of EPs during the COVID-19 pandemic compared to the period before the pandemic [16–18]. Authors reported delayed presentation of EPs along with an increase in blood loss in patients treated surgically [19, 20]. The exact cause of this trend is yet to be confirmed, but the disruption of primary healthcare, women's fear of exposure, and reduced medical checkups together could be the underlying explanation [19]. Lastly, a study from Brazil noted that SARS-CoV-2-positive patients with EP should be treated surgically since MTX could reduce immunity, and the pulmonary disease itself represents a contraindication for this treatment [21]. In the study regarding the hysteroscopic treatment of cervical pregnancies, the authors noted shorter hospital stays, reduced blood loss, and shorter time needed for recovery [22]. Even though our study has the crucial limitation of only two cases, the advantages of the hysteroscopic approach should be emphasized even more during the COVID-19 pandemic.

Conclusion

We have successfully treated two asymptomatic, clinically stable patients with IP of gestational age less than 10 weeks by ultrasonography and serum βhCG < 7,000 mIU/mL. With numerous advantages of the minimally invasive approaches to EP treatment, large, multicenter, and comparative studies should be performed to further investigate the place of hysteroscopy as a single treatment method for IP. Moreover, during the ongoing pandemic, where we experience the disruption of the health system and observe the trend of EPs that require emergency surgical treatment, the place and advantages of the minimally invasive approach should be highlighted. Consequences and trends observed during the COVID-19 pandemic correlate with the importance of timely diagnosis of EPs, the benefits of a minimally invasive approach in their treatment, and epidemiologically justified shorter hospital stays.

Conflict of interest

The authors declare no conflict of interest.

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CASE REPORT

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Pneumonia caused by coagulase-positive methicillin-resistant *Staphylococcus aureus*

Pneumonija izazvana koagulaza-pozitivnim meticilin rezistentnim *Staphylococcus aureus*-om

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Abstract

Introduction. *Staphylococcus* (*S.*) *aureus* is one of the most omnipresent and dangerous human pathogens, whose main characteristic is the production of the enzyme coagulase. This characteristic serves to identify and assess the pathogenicity of the bacteria. In addition to skin infections, endocarditis, osteomyelitis, and infectious arthritis, it is a common cause of pneumonia both in children and adults**. Case report.** We described a case of a 65-year-old woman with a dry cough and malaise with patchy areas of consolidation on the chest X-ray and "ground-glass" opacity with bronchial wall thickening and unilateral mediastinal lymphadenopathy on chest computed tomography imaging. Methicillin-resistant *S. aureus* was isolated from the bronchoalveolar aspirate taken during bronchoscopy. The woman was empirically treated with azithromycin, and later, based on the antibiogram findings, azithromycin was replaced with meropenem, after which her health improved. **Conclusion.** We presented a rare case of pneumonia with unconvincing symptomatology and laboratory and radiological findings. Paying more attention to such cases in the future is crucial, especially to the use of antibiotics to which staphylococci are increasingly developing resistance.

Key words:
antibiotics; bronchoscopy; coagulase; methicillin resistance; pneumonia; staphylococcus aureus.

Apstrakt

Uvod. *Staphylococcus* (*S.*) *aureus* je jedan od najprisutnijih i najopasnijih humanih patogena čija je glavna karakteristika stvaranje enzima koagulaze. Ova karakteristika omogućava identifikaciju i procenu patogenosti bakterije. Pored kožnih infekcija, endokarditisa, osteomijelitisa i infektivnog artritisa, čest je uzročnik pneumonije kako kod dece tako i kod odraslih. **Prikaz bolesnika.** Prikazana je žena starosti 65 godina, sa tegobama u vidu suvog kašlja i malaksalosti, sa rentgenskim nalazom mrljastih polja konsolidacije i promenama tipa „mlečnog stakla" sa zadebljanjem bronhijalnog zida i jednostranom medijastinalnom limfadenopatijom na snimku grudnog koša dobijenom kompjuterizovanom tomografijom. Meticilin rezistentni *S. aureus* izolovan je iz bronhoalveolarnog aspirata uzorkovanog tokom bronhoskopije. Bolesnica je empirijski lečena azitromicinom, a kasnije, na osnovu rezultata antibiograma, isključen je azitromicin i uveden meropenem posle čega je usledilo poboljšanje zdravstvenog stanja bolesnice. **Zaključak.** Prikazan je redak slučaj pneumonije sa neubedljivom simptomatologijom, laboratorijskim i radiološkim nalazima. Neophodno je posvetiti više pažnje ovakvim slučajevima ubuduće, posebno na upotrebu antibiotika na koje stafilokoke sve više razvijaju rezistenciju.

Ključne reči:
antibiotici; bronhoskopija; koagulaza; meticilin, rezistencija; pneumonija; staphylococcus aureus.

Introduction

Coagulase-positive *Staphylococcus* (*S.*) *aureus* is one of the most omnipresent and dangerous human pathogens, both because of its virulence and capability to develop antibiotic resistance [1]. *S. aureus* is the only species of the genus *Staphylococcus* that produces coagulase, and this characteristic is used as one of the criteria for identifying and assessing staphylococcal pathogenicity [2]. Pneumonia, one of the diseases caused by *S. aureus*, is not so common except in patients on corticosteroid therapy, those who already have influenza, or those with chronic bronchopulmonary diseases.

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Pneumonia can occur as a primary lung infection or by the hematogenous spread of a pathogen (as an intravenous catheter infection, endocarditis, or soft tissue infection), as well as a consequence of intravenous drug administration [1]. According to the data in the literature, it is interesting that in some populations, *S. aureus* is the most common cause of hospital-acquired pneumonia (HAP), defined as an event that happens more than 48 hours after admission to the hospital [1, 3, 4]. Methicillin-resistant *S. aureus* (MRSA) is becoming the pathogen that all the more often causes other forms of pneumonia: community-acquired pneumonia (CAP), healthcare-associated pneumonia (HCAP), and ventilator-associated pneumonia (VAP) [5]. Nowadays, according to some authors, community-associated methicillin-resistant *S. aureus* (CAMRSA) is the newest menace to patients hospitalized with pneumonia. The Center for Disease Control and Prevention (CDC) has set the following criteria for distinguishing CAMRSA from other hospital strains: a) a diagnosis of MRSA made in an outpatient setting or culture positive for MRSA 48 hours after hospital admission; b) no evidence of MRSA infection or colonization in the patient's medical history; c) for the last year the patient has not been hospitalized, stayed in a nursing home, received hospice care, underwent dialysis or surgery; d) the patient does not have a permanently applied catheter or another medical device that passes through the skin into the body [6]. As stated by multiple authors, the number of hospitalizations due to *S. aureus* pneumonia decreased by 24% from 2009–2012 in the USA, largely driven by a 19% decrease in MRSA pneumonia [7]. We herein described a case of a woman who came to our hospital with specific radiological findings, in whom *S. aureus* was isolated during hospitalization and later confirmed that the pneumonia was caused by MRSA.

Case report

A 65-year-old woman came to our hospital complaining of a dry cough and malaise lasting for several days. During the examination, on admission, the patient was afebrile, blood pressure was 120/70 mmHg, and auscultation breathing noise was weakened on both sides without any accompanying pathological findings. The patient submitted a chest X-ray, done outside of our hospital, showing bilateral patched areas of consolidation, predominantly basal without pleural effusion (Figure 1A), on the basis of which the attending physician decided to hospitalize the patient. Laboratory findings were within normal range, except for C-reactive protein (CRP), which was elevated to 100 mg/mL. Sputum was sterile. Tumor markers like neuron-specific enolase (NSE), carcinoembryonic antigen (CEA), and the cytokeratin 19 fragmentation antigen (CYFRA 21-1) were also negative. The patient was prescribed azithromycin for seven days, after which the level of CRP was still increased. We then decided to do a chest high-resolution (HR) computed tomography (CT) HRCT, which showed bilateral diffuse patchy consolidations and subtle "ground-glass" opacities with predominantly subpleural and peribronchial distribution with present bronchial dilatation and wall thickening in the abnormal region, unilateral mediastinal lymphadenopathy without pleural effusion (Figure 1B - 1H). Since the diagnosis was not discernible, it was decided to perform a bronchoscopy, during which a bronchoaspirate was taken, seeded on an appropriate medium where a coagulase-positive *S. aureus* was later isolated. According to the antibiogram, the meropenem was administered intravenously for seven days. After the application of meropenem, the radiological changes were withdrawn, and the CRP decreased to 20 mg/mL, after which the patient was released for home treatment. During hospitalization, according to the protocol for sepsis, we made an additional cardiological ultrasound examination and a Doppler ultrasound of the legs, both of which were within normal range.

Discussion

In recent years, the prevalence of MRSA-induced pneumonia has declined among hospitalized patients in the United States. This fact is accompanied by mortality improvement and a shortened hospital stay. MRSA pneumonia prevalence constantly reduced from 2009 (75.6 cases per 100,000 releases) to 2012 (56.6 cases per 100,000 releases) [7]. Further along, some authors believe that CAP caused by *S. aureus* has a high mortality rate, around 16.6% according to their research [8], but, on the contrary, the mortality rate from MRSA pneumonia decreased from 7.9% to 6.4% between 2009–2012 [7]. In addition to affecting infants and children [9], MRSA pneumonia is becoming more common in the elderly population. Our patient was 65 years old at the time of diagnosis; similar average data was obtained by different authors who reported that the median age for HAP was 68 years, and for HCAP, 74 years [10]. Dry cough and malaise were the main symptoms of our patient without the accompanying fever. Data from some studies show that cough is the most common symptom in 86% of cases, shortness of breath in 79%, spu-

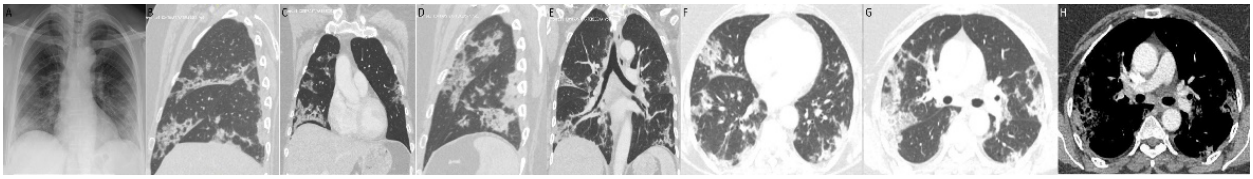

Fig. 1 – A) Chest X-rays of the presented patient: bilateral patchy areas of consolidation predominantly basal without pleural effusions; B-F) High-resolution computed tomography (HRCT), various radiological sections: bilateral diffuse patchy consolidations and subtle "ground-glass" opacities with the predominantly subpleural and peribronchial distribution; G) HRCT: bronchial dilatation and wall thickening in inflammatory regions; H) HRCT: unilateral mediastinal lymphadenopathy, no pleural effusion.

tum production in 64%, while fever is recorded in 50%, and weakness in 43% of respondents [11]. In one study, on chest examination, doctors noticed the lower respiratory sound and sporadic rales at the base of both lungs, similar to our findings [12]. Laboratory findings were inconclusive, except for elevated CRP. A slight increase in leukocytes with the predominance of neutrophils in the proportion of 94% represented a significant laboratory result that indicated a bacterial infection. CRP and procalcitonin were also elevated at 14.2 mg/dL and 26.6 ng/mL, respectively [12]. In our patient, bacteria were not isolated by microbiological treatment of sputum; contrary to some studies where MRSA was isolated from sputum, we used bronchoalveolar lavage, and all our results were confirmed in blood culture [13, 8]. Tumor markers were negative; furthermore, we could not find any research about the interconnection between MRSA pneumonia and changes in the levels of tumor markers. Radiological findings showed patched areas on both lungs; a similar description was given by other authors [13]. One retrospective study described the percentage of individual changes in X-ray findings in MRSA pneumonia – the most commonly described were cavitation/necrosis (43.5%), lobar pneumonia (37.5%), multilobar pneumonia (31.2%), effusions/empyema (31.2%), and diffuse patchy (25%) infiltrates [14]. In another case report, authors described CT findings as multiple consolidations in bilateral upper and lower lobes [13]. According to data from another retrospective study, "ground-glass" attenuation was the most described finding on CT (79.4%), compared to bronchial wall thickening (60.3%), consolidation (58.8%), bilateral pleural effusion (51.5%), and bilateral lymph node enlargement (64.7%). The changes most commonly affected the lower lung fields [15]. Most of the above-described changes were present on the CT of our patient. Bronchoscopically, we took an aspirate from which MRSA was isolated after cultivation, thus making the right diagnosis. Searching the literature, we came across only one study where bronchoscopy was used to take a bronchoalveolar lavage from which *S. aureus* was isolated in more than 100,000 bacteria per mL; thus, this finding was considered significant for diagnosis [16]. Prior to diagnosing MRSA pneumonia, based on the clinical picture and radiological findings, the patient was given azithromycin intravenously for seven days; the same antibiotic was empirically included by doctors in one local hospital, assuming it was CAP [12]. Based on the results of the antibiogram, we decided to change the antibiotic and administer meropenem. However, according to the results of the antibiogram and in accordance with their experience, some authors opted for linezolid [13], whereas others noted a significant decrease in CRP using tigecycline empirically [12]. According to new guidelines for empiric treatment of MRSA pneumonia, the treatment should include vancomycin or linezolid [17], although a few authors prefer linezolid because of its ability to inhibit bacterial toxin production. A randomized trial showed superiority in clinical outcomes but not in mortality after linezolid administration compared with vancomycin in HAP or HCAP MRSA pneumonia [18]. Some authors also believe MRSA pneumonia should be treated with vancomycin, linezolid, or ceftaroline in resistant cases [19]. Our patient was hospitalized for more than two weeks, while according to some researchers, the length of hospital stay for MRSA pneumonia was between 6.9–7.8 days [7].

Conclusion

We have presented a rare case of pneumonia caused by MRSA, with few symptoms and unconvincing laboratory and radiological findings. Timely thinking, adequate diagnostics, and antibiotic therapy will reduce morbidity and mortality rates of this type of pneumonia. A crucial problem today is the staphylococcal strain which is becoming increasingly resistant to the antibiotic therapy applied according to therapeutic protocols. Therefore, in the future, more rational use of antibiotics in treating infectious conditions must be taken into account.

Conflict of interest

The authors declare no conflict of interest.

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Efficiency of a 3-week multicomponent rehabilitation on improving the function in a patient with Friedreich's ataxia – a case report

Delotvornost tronedeljne višekomponentne rehabilitacije na poboljšanje stanja kod bolesnika sa Fridrajhovom ataksijom

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Abstract

Introduction. Friedreich's ataxia (FA) is an autosomal recessive neurodegenerative disease. Ataxia, as the cardinal symptom, affects the trunk, with swaying, imbalance, and falls, as well as the limbs, with increasing difficulty in activities of daily living. Physical therapy has been recognized as a means of managing physical symptoms and maximizing function in affected persons. To our knowledge, there are no studies that have evaluated the effectiveness of proprioceptive neuromuscular facilitation (PNF) stabilization techniques in the rehabilitation of patients with such a diagnosis. **Case report.** We present a 26-year-old female with FA who had severe truncal and limb ataxia, speech difficulty, and poor walking ability. During the three-week rehabilitation, an individually tailored physical therapy program based on PNF stabilization techniques was applied. The implemented rehabilitation program resulted in an overall functional improvement. The reduction in ataxia was registered according to the Scale for the Assessment and Rating of Ataxia (SARA). The Functional Independence Measure (FIM) instrument – a component of locomotion, revealed greater independence in walking. **Conclusion.** A rehabilitation program based on PNF stabilization techniques may reduce ataxia and improve walking ability in patients with FA.

Key words:
friedreich ataxia; physical and rehabilitation medicine; treatment outcome.

Apstrakt

Uvod. Fridrajhova ataksija (FA) je autozomno recesivna, neurodegenerativna bolest. Ataksija kao glavni simptom zahvata trup, uz njihanje, nestabilnost i padove, kao i ekstremitete, uz sve teže obavljanje aktivnosti svakodnevnog života. Fizikalna terapija se prepoznaje kao sredstvo za tretman fizičkih simptoma i postizanje maksimalne funkcije obolelih osoba. Prema našim saznanjima, ne postoje studije koje su ocenjivale korisnost tehnika stabilizacije proprioceptivne neuromišićne facilitacije (PNF) u rehabilitaciji bolesnika sa tom dijagnozom. **Prikaz bolesnika.** Prikazujemo ženu staru 26 godina sa FA koja je imala težak oblik ataksije trupa i ekstremiteta, teškoće u govoru i slabu sposobnost hodanja. U toku tronedeljne rehabilitacije primenjen je individualno prilagođen program fizikalne terapije, baziran na tehnikama stabilizacije PNF. Primenjeni rehabilitacioni program rezultirao je opštim funkcionalnim poboljšanjem. Smanjenje ataksije je registrovano Skalom za procenu i ocenu ataksije (*Scale for the Assessment and Rating of Ataxia* – SARA). Instrumentom za merenje funkcionalne nezavisnosti (*Functional Independence Measure* – FIM) – komponenta kretanja, pokazana je veća nezavisnost u hodu. **Zaključak.** Rehabilitacioni program zasnovan na tehnikama stabilizacije PNF može smanjiti ataksiju i poboljšati sposobnost hodanja kod obolelih od FA.

Ključne reči:
ataksija, fridrajhova; medicina, fizikalna i rehabilitacija; lečenje, ishod.

Introduction

Friedreich's ataxia (FA) is a hereditary disease with progressive neurological features named after the German physician Nikolaus Friedreich who first described it in 1863 [1]. The first symptoms usually appear in childhood or adolescence, and instability is most often reported as the first main symptom [2]. The prevalence of this rare disease ranges from one case in 20,000 in southwestern Europe, while in northern and eastern Europe, the prevalence is lower: one case in 250,000 [3]. Traditionally viewed as a neurodegenerative disease, FA patients also develop cardiomyopathy, scoliosis, diabetes mellitus, and other manifestations [4]. The main manifestations of FA include symptoms of afferent and cerebellar

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ataxia, central muscle weakness, speech disorder, swallowing problems, inability to maintain balance and proprioception, and vibration sensation disorders [5]. Walking disorders start early and manifest as unstable gait. Limb ataxia manifests as a lack of precise movement, slowness of movement, intentional tremors, and a coordination disorder. Basic activities of daily living (ADLs) become difficult due to ataxia. Usually, most patients are wheelchair-assisted by the third decade of life [3]. There is no approved disease-modifying therapy for the treatment of this disease [6]. Physical therapy is recommended to alleviate symptoms and improve motor function. The evidence published so far indicates that rehabilitation is effective in reducing ataxia and improving balance, mobility, and function [4, 7, 8]. A wide range of physical therapy interventions is effective in treating the symptoms and controlling the progression of this disease. In the rehabilitation of ataxia, static and dynamic balance exercises, gait exercises, coordination, and exercises to increase proprioceptive awareness are implemented [9]. Most studies have shown a reduction in ataxia symptoms due to the use of balance exercises [10, 11], but there is insufficient high-quality evidence to form clear recommendations and guidelines for clinical practice. We present the successfully performed multicomponent physical therapy intervention involving proprioceptive neuromuscular facilitation (PNF) stability exercises in patients with FA that could improve the clinical practice of such a rare disease.

Case report

We present a 26-year-old female complaining of generalized ataxia, unable to walk independently. FA was diagnosed when she was 21 (in 2016). The family history was negative. Symptoms gradually progressed to severe gait instability. A year after the diagnosis, she started using a walker. She did not report any further worsening of ataxia symptoms after the SARS-CoV-2 infection in November 2020. Mobilizing independently indoors, she reached for furniture to provide support when walking; she had several falls. She reported walking independently on the stairs in the house, holding onto the handrail with both hands. She was not socially active regularly due to a walking disability. Over the last year, symptoms progressed to serious gait instability: she became dependent on using a wheelchair for distance mobility. Carnosine supplementation was performed. She described a regular home exercise program that considered muscle strengthening exercise with 1 kg weighted cuff resistance and a few min of cycling on a stationary bike. Family support was present. In October 2021, she was admitted to the Institute for Physical Medicine, Rehabilitation, and Rheumatology “Dr. Simo Milošević” in Igalo, Montenegro for a regular three weeks of rehabilitation provided by the healthcare system once a year.

Assessment

On admission, an observation revealed kyphoscoliosis and hand muscle wasting. There were no signs of other significant muscle wasting, tingling, or paresthesia, and no abnormalities in hearing or vision. Manual muscle testing (MMT) was used for assessing general muscular strength [12, 13], and the score was 4/5. She had dysmetria, dysdiadochokinesia, and intentional tremor bilaterally. Many words were difficult to understand. Clinical evaluation was performed through the Scale for the Assessment and Rating of Ataxia (SARA). The use of the SARA scale is valuable for assessing ataxia and rehabilitation planning [14, 15]. SARA test scored 24/40. There was no evidence of increased muscle tone. Straight leg raise (SLR) test was positive, left 60°, right 50°. She was independent in the sit-to-stand movement. Independent sitting balance in supported sitting was present, with intermittent sway while she was sitting unsupported. In a wide stance, she stood independently for less than 10 sec. Romberg was positive. She was able to walk a short distance with the support of two persons or with a walker and the support of one person. The Functional Independence Measure (FIM) scale [16, 17] component of locomotion was rated 8/14. Overall, she did not require any assistance to perform basic ADLs: eating, dressing, and personal hygiene.

Intervention

We implemented a neurorehabilitation program aimed to improve ataxia, stability, and walking ability to prevent and slow complications of primary illness (Figures 1–4). The rehabilitation program considered the following exercise sessions: manual massage twice a day for 45 min and occupational therapy once a day for 15 min. During rehabilitation, an individually-tailored, problem-solving physiotherapy approach consisting of PNF stabilization, walking, and proprioceptive exercises was conducted (Table 1). To prevent and slow the sequelae of primary illness, isotonic muscle strengthening exercises with optimal manual resistance were applied.

Fig. 1 – Stabilizing reversal in side-lying in a patient with Friedreich’s ataxia.

Fig. 2 – Rhythmic stabilization of a patient with Friedreich's ataxia in prone elbows support.

Fig. 3 – A patient with Friedreich's ataxia: two-wheeled walker support. Controlled stepping.

Fig. 4 – Frenkel exercises over slide board in supine.

PNF therapy intervention was carried out following the philosophy of the method and principles of facilitation, such as resistance, approximation, stretching, movement patterns, manual, verbal, and visual contact, correct position, and the therapist's body position [18]. PNF techniques, such as stabilizing reversal and rhythmic stabilization, may be applied to promote trunk stability within the different postures that constitute the developmental sequence [19]. Generally, improvements in balance have been attributed to PNF stabilizing techniques [18, 20–22]. To promote stability, as balance disorder is one of the main manifestations of FA, we applied the PNF techniques stabilizing reversal and rhythmic stabilization in different positions: half-ring sitting, ring sitting, side-lying, side sitting with and without arms support, prone on elbows, quadruped, and sitting with and without foot support. With rhythmic stabilization, the subject tries to maintain position while the therapist provides perturbances without any movement, while in stabilizing reversal, small movement is allowed. To promote stability in kneeling and standing, approximation was given through both sides of the pelvis equally and directed downward. She practiced sitting steady without leg support for 30 sec with her arms outstretched forward. In a half-ring sitting position and sitting without foot support, we performed reaching out for objects from different angles.

Table 1

Physical therapy program[1]

Problem	Physical therapy interventions
General muscle strength and endurance	Abdominal curls, prone fly. Bridge with approximation over knees and with isometric hold. Isotonic strengthening exercises with manual resistance for upper extremity and lower extremity muscles, 1–2 series, 10 repetitions.
Postural control	To promote stability, we applied PNF techniques rhythmic stabilization and stabilizing reversal in side-lying (Figure 1), sitting with and without foot support, half-ring sitting, side sitting with and without hand support, and prone on elbows (Figure 2), and quadruped. To promote stability in kneeling and standing, approximation was given through both sides of the pelvis equally and directed downward. We practiced sitting steady without foot support for 30 sec with arms outstretched forward. In half-ring sitting and sitting without foot support, we performed reaching out for objects from different angles.
Walking	Walking with parallel bars, physiotherapist's support, and instructions, just a few meters at first. At the end of the second week, we started walking with two-wheeled walker support. Controlled stepping was practiced under visual control (Figure 3). We gradually extended the walking distance.
Proprioceptive awareness	Frenkel exercises over the slide board in a supine (Figure 4) and, in progression, in a sitting position. In sitting, she also placed her foot in marked spots on the floor. Finger chase exercise for the upper extremity.
Reduced flexibility	Hamstrings stretching, hold-relax PNF technique, and from the third week active stretching in sitting.

[1] Problem-oriented physiotherapy program based on proprioceptive neuromuscular facilitation (PNF) stabilization techniques also included Frenkel exercises, walking exercises, and strengthening exercises.

Table 2

Scale for the Assessment and Rating of Ataxia (SARA) and Functional Independence Measure (FIM) test rating, before and after rehabilitation[2]

Outcome measure	Before rehabilitation	After rehabilitation
SARA test score	24/40	20/40
Gait	7/8	6/8
Stance	4/6	3/6
Sitting	1/4	1/4
Speech disturbance	4/6	4/6
Finger chase	1/4	1/4
Nose-finger test	2/4	1/4
Fast alternating hand movements	2/4	2/4
Heel-shin slide	3/4	2/4
FIM test (locomotion) score	8/14	10/14
Walk	3/7	5/7
Stairs	5/7	5/7

[2] SARA score before and after rehabilitation showed a decrease in sub-items scores of gait, stance, and tremor amplitude with nose-finger test and heel-shin slide. FIM score before and after rehabilitation showed improvement in the sub-item score of the walk.

Outcomes

At the final assessment, the patient reported greater endurance with supported standing and walking. SLR test was positive: left 70°, right 65°. SARA test scored 20/40: examination showed a decrease in sub-items scores of gait, stance, and tremor amplitude with the nose-finger test and heel-shin slide (Table 2). She was able to walk with walker support and supervision. FIM scale rating of 10/14 revealed improvement in locomotion.

Discussion

FA is a progressive neurological disease with several different manifestations. The current treatment of that genetic disorder is directed toward all individual manifestations. Science is constantly searching for effective disease-modifying therapy, and meanwhile, rehabilitation remains the base of treatment that controls symptoms and slows the progression of the disease [6]. In various neurological disorders, including FA, intensive rehabilitation in the institution

has been effective in improving patient function [23]. Improvement in balance may occur after 3 weeks, and improvement in ataxia requires at least 4 weeks of rehabilitation. In addition, multicomponent inpatient rehabilitation has greater effects than home exercise programs alone [4]. The home exercise program cannot achieve or maintain the benefits of multi-segment rehabilitation [6]. That emphasizes the importance of inpatient rehabilitation. However, a three-week rehabilitation is just a "drop in the ocean" for patients with FA [24]. To achieve the maximum level of functioning, it would be helpful to provide inpatient rehabilitation more than once a year. There are very few patients with this diagnosis, and this would not significantly burden the healthcare system.

As part of rehabilitation, there are a large number of therapeutic interventions that differ in type, intensity, and duration. However, in a systematic review, Milne et al. [8] state that the evidence suggesting that rehabilitation improves function, mobility, balance, and ataxia is consistent. Physical therapy interventions enable the improvement of muscle flexibility and strength, postural control, and movement dexterity. By acting on the individual manifestations of this neurological disorder, physical therapy alleviates the effect of ataxia on patient function [3]. One of the main goals of neurological rehabilitation is to improve gait function [25]. Balance exercises and coordination exercises are especially useful for the functional recovery of the patient. There are no available data from high-quality randomized trials on the effectiveness of physical therapy interventions. Marquer et al. [26] stated that the intensity, duration, and content of the rehabilitation programs would have to be better defined.

During a three-week rehabilitation, we implemented an individually tailored, problem-oriented physiotherapy program based on PNF stabilization techniques. Outcome measures in our study showed improvement in overall functional performance. Such improvement may be due to specific stabilization techniques. Improved trunk stability had an influence on the reduction of upper limb tremors. Applied techniques resulted in better walking ability and diminished ataxia.

PNF stabilization concepts are effective in the treatment of postural control in stroke rehabilitation [18, 22]. As far as we know, there are no studies that have evaluated the effectiveness of PNF stabilization techniques in patients with FA, so the results of our research are worthy of attention. One case report limits the generalization of our findings. Our case report indicates the effectiveness of a three-week multicomponent rehabilitation, but long-term follow-up of the patient was lacking. The systematic review from 2017 emphasized that it is not clear how long the effects of rehabilitation are maintained [8]. Future research should consider monitoring the functional status of patients after discharge to gain insight into the duration of rehabilitation outcomes.

Although the conclusion is based only on one case, this paper can provide preliminary guidance for physiotherapists in treating individuals with FA.

Conclusion

The findings of this study provide evidence that an individually tailored rehabilitation program based on PNF stabilization techniques can reduce ataxia and improve functional status in patients with FA. The stabilization techniques of the PNF approach should be considered and included during the short-term multicomponent rehabilitation of these patients.

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Plague epidemics in the southern region of the Habsburg Monarchy in the XVIII century – fear, prejudices, and consequences

Epidemija kuge u južnom regionu Habsburške monarhije u XVIII veku – strah, predrasude i posledice

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disease outbreaks; history of medicine; history, 18th century; plague; serbia.

Ključne reči:
epidemije; istorija medicine; istorija, 18. vek; kuga; srbija.

Introduction

In the history of human civilization, the plague, an acute infectious zoonotic disease, represented one of the most dangerous epidemic diseases with an extremely high mortality rate. Thucydides described the plague that ravaged from 435 to 430 before Christ (BC) [1]. The plague struck again in 166 *anno Domini* (AD) and from 215 to 266 AD in the Roman Empire. The "Justinianic Plague" struck the Byzantine Empire from 531 to 580 AD. The plague epidemic was quite intense during the 40s and 50s of the 14th century. The plague is caused by the *Yersinia pestis* bacteria, transmitted from rodents to humans by fleas, and when an infected person has the so-called lung plague (pneumonic), it can also be transmitted through the air. In addition to this type, the most common is the bubonic plague, which got its name from the word buboes, meaning swollen lymph glands. The incubation period for this type of plague is between two and five days from the appearance of the first symptoms – chills, high fever, and in some cases, high pulse rate and hypertension. Soon after, more symptoms appear, the lymph nodes become swollen, the chills and high fever continue, and, at this stage, extreme headaches become quite frequent. This is followed by coughing, which at a certain point turns into coughing out blood, and the final stage of the disease is sepsis, which ends in death, often only 48 hrs after its beginning, although the illness may last longer. When it comes to treatment, it is crucial to begin as early as possible, preferably in the first 24 hrs, by giving the patient streptomycin. Moreover, implementing isolation measures quickly, especially in cases of pneumonic plague, is essential [2–4].

There were several plague outbreaks in the Habsburg Monarchy during the seventeenth and eighteenth centuries, usually not on the entire territory, but with catastrophic outcomes in the regions where it did appear. After the Battle of Vienna (1683–1699), when the borders of the Monarchy were expanded far to the south and the east, the plague broke out as early as 1703 and 1704 in the eastern regions, widely among the Serbian population. A more extensive plague epidemic broke out in 1708 in the south of the state, in the region of Bačka (between the rivers Tisa and Danube). Only five years later, the plague devastated the southern regions of the Monarchy once again, with the last recorded time being in Vienna, at which time 13,407 people died. That same year, it also hit Styria, Carniola, and Carinthia. During the same period, 35,834 people died from the plague in Prague. This was the last plague epidemic that hit Vienna, and it was recorded that the plague killed 10% of the population on the territory of the Kingdom of Hungary. In the period that followed, there were sporadic plague outbreaks of varying intensity, especially from 1738 to 1744, while in 1740, it spread over almost the entire territory of the Kingdom of Hungary. The situation became even worse with the outbreak of the War of the Austrian Succession (1740) because the army spread and transmitted the disease more easily. The war was fought in the Lands of the Bohemian Crown, with Silesia at the center of the fighting, and then the retreat to Moravia brought the dise-

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ase there. The enormous number of deaths during this epidemic compelled the state to enforce improved measures and intensify border security in order to prevent the plague from spreading to the Monarchy [5–8].

For the purposes of this study, all available literature was used, both published and unpublished sources dealing with this subject in Serbian, Latin, and German. The interpreted unpublished sources can be found in the Serbian Academy of Sciences and Arts Archives in Sremski Karlovci, in 'A' and 'B' funds, which also include documents based on the correspondence between the metropolitans of Karlovci, state representatives, and the Royal Court (fund 'A'), as well as written documents created on the territory of Srem (fund 'B'), hit by an epidemic outbreak in 1795/96. While researching this Archive, several dozens of documents were discovered concerning efforts to prevent the plague, the metropolitan's instructions to close churches, regulations regarding sanitary measures, numerous data on implementation oversights, as well as the reactions of the people. Based on such data, it was easier to form a picture of the situation in Srem at the time of the last great plague epidemic in the southern part of the Habsburg Monarchy. Furthermore, the plague managed to enter the Banat region for the first time in 1737. It caused damages in the city of Temeswar (*Timişoara*, in Romanian) [9]. In addition to the excellent archival material, published sources were also used in writing this study, including the work written by Dr. Franz von Schrauder, a physician who was in Srem at the time of the epidemic and who described its development [10].

The places with the highest number of patients with plague are shown in Figure 1.

The sanitary cordon and the battle against fear and prejudices among the general population

For the most part, the plague was transmitted to the Habsburg Monarchy from the Ottoman Empire, where the epidemic never seized completely but rather only abated [11–13]. Just how much this influenced the decision to implement health regulations in the Habsburg Monarchy is demonstrated by the fact that all traffic to the Ottoman Empire was forbidden during the time of the epidemic, in accordance with "the Plague patent" *(Pestpatent)* of June 25, 1710, and *Pestordnung* of 1713, in order to prevent the spread of the disease. Moreover, the majority of the work on health laws was initiated precisely under the influence of the horrific devastation incurred by the plague in 1738–1744. By this, the military frontier, a specific region that constituted a large part of the Austrian army and extended from the Adriatic Sea to Transylvania, also served as a sanitary cordon, or in other words, a heavily guarded territory established in 1553 to prevent attacks from the Ottoman Empire and the epidemic from spreading inland [14, 15]. Border control was implemented by placing a series of guard stations along the frontier which were close to each other, enabling the people inside to see the next station with their naked eye and thus preventing any attempts of illegal border crossing. The border regiments had special medical service personnel, and all hospitals were under the supervision of military surgeons. Through their trustworthy people, special sanitary commissions gathered information on the state of health in the Turkish border regions regularly, and in addition, there was also a special health police. A series of laws issued from 1731 to 1740 regulated the construction of quarantines – buildings for isolat-

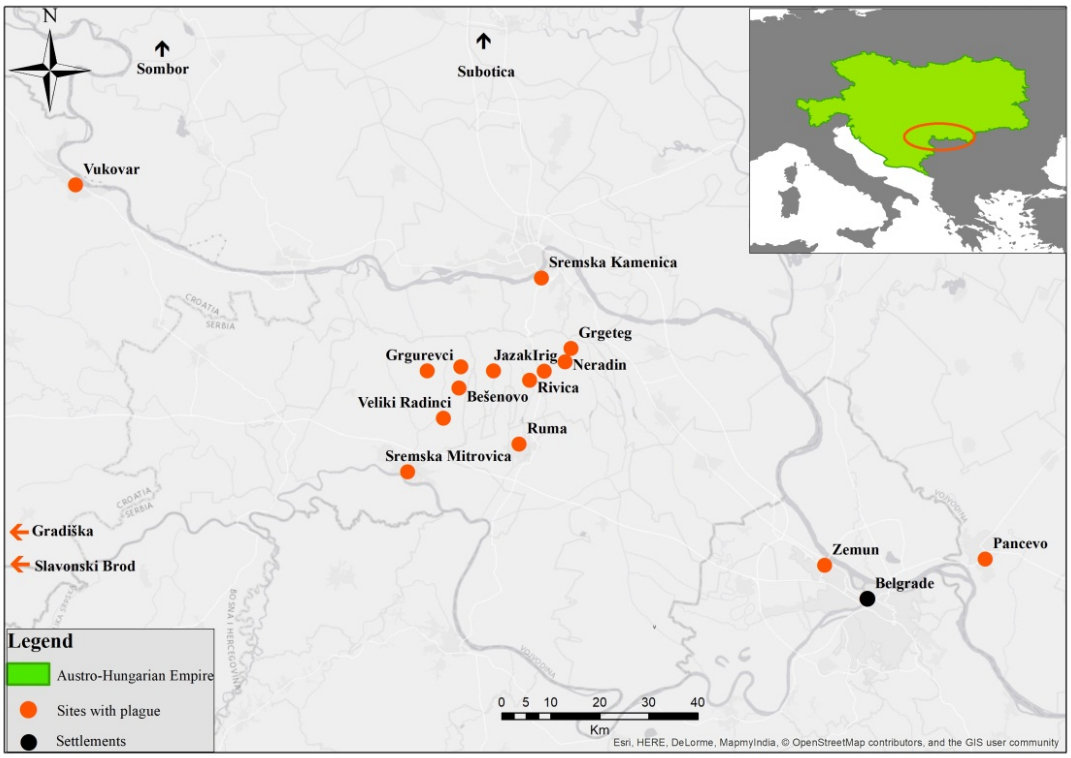

Fig. 1 – Places with the highest number of cases of plague.
Source: The map made by the authors.

ing individuals crossing from one state to the other, in detail. Depending on the presence of the epidemic in the neighboring regions, the people, together with their goods or personal items, had to spend either a specific amount of time in these buildings or until the healthcare workers were convinced there was no infection. At times when there was no plague epidemic in the neighboring regions of the Ottoman Empire, the quarantine lasted 21 days, which was considered the first stage of defense and applied to everyone.

When there was an ongoing plague epidemic in the distant regions of the Ottoman Empire, the second stage of defense was implemented, according to which the quarantine lasted 28 days. During an epidemic outbreak in the border region, the quarantine lasted 42 days for individuals and 56 days for goods, which was considered the third stage of defense. In the quarantine area, the trade goods and personal items were unpacked, smoked, and exposed to the sun, which was the standard procedure for disinfection. The construction of quarantine buildings began in 1731 using friable materials, but most of the work was done during the time of Maria Theresa when the buildings were erected using solid materials. Crossing borders in the southern part of the Monarchy, especially during the epidemic, was enabled only in places with quarantines (the most significant were Pančevo, Zemun, Sremska Mitrovica, Slavonski Brod, and Gradiška). The defense system was improved in 1753 when the Royal Health Commission was formed and then again in 1755 when the *Ordo pestis* was issued, which was then supplemented in 1764, all following comprehensive state reforms defined by Professor Gerard van Swieten in cases pertaining to health issues. These reforms prohibited (1745) the work of quack doctors, and, as of August 3, 1756, the deceased were not allowed to be buried until the surgeons examined the body and established the cause of death. Furthermore, a decree was issued according to which the deceased were not to be buried at least 36 hrs after the moment of death [16, 17].

The implementation of these measures prevented the plague from spreading outside the border region of the Monarchy in 1762 and contained the infection to six villages, thus proving its efficiency [18]. Although the southern neighbors of the Habsburg Monarchy had another plaque outbreak the following year, it did not spread to its regions due to the implementation of the decision to prohibit traffic at borders without quarantine facilities. The Serbian Church within the Monarchy played a very significant role because its archbishop requested, in the form of a pastoral letter, that the people cease all communication with the Ottoman Empire [19]. In 1770, based on the acquired experience and the implemented regulations, van Swieten wrote the General Norm of Health Service, according to which doctors, surgeons, and pharmacists were required to have a university degree and whose goal was to increase the level of health awareness. In addition, anyone who violated the plague prevention regulations was sentenced to death by hanging, and the same sentence applied to all accomplices and officers who enabled individuals to evade the quarantine altogether or leave before the stipulated period, as well as individuals who gave false statements regarding the origin of the goods and the people. The efficiency of these regulations was obvious because their implementation in the second half of the eighteenth century kept the plague epidemic along the border of the Monarchy with the Ottoman Empire at bay. Most of these regulations were repeated in the so-called Chenot Norm issued in 1785, which also dealt with measures for plague prevention, and only the recommended quarantine period was shortened [20, 21].

New regulations, issued during the Joseph II reign, applied to all the nations of the heterogeneous Monarchy and all levels of society. However, the reaction to this set of laws varied. Regulations regarding the burial of the deceased were met with the greatest disapproval, mostly among the uneducated or barely educated population (peasants), but also the clergy, and this was the case with the majority of the Serb and Vlach population in the southern regions of the Monarchy. Although the provisions prescribed the period between the burial of the deceased and the time of death, the appearance of the grave sites, which had to be outside the town borders, fenced and orderly, not allowing the deceased to be carried through town in open caskets, and so on, undoubtedly represented a set of progressive civilizational norms, from the aspect of customs, they nevertheless violated established practices. The Serb population, most of whom lived precisely in the Military Frontier, met all of the decrees with disapproval. At the time when these important health regulations were issued, Archbishop Pavle Nenadović was the ecclesiastical and secular leader and the Serbian Orthodox Metropolitan of Karlovci (1749–1768). He was an educated individual who, sometimes on his initiative and sometimes the state's, attempted to eradicate every type of superstition, both the negative pagan burial customs and rituals and the unhealthy lifestyle. Following his death, the state took over the initiative through its Regulations of 1770, 1777, and the Declaration of 1779. However, the resistance of the people was not broken, which was evident during the plague epidemic outbreak in 1795/96 in Srem, a border region with the Ottoman Empire, from where the disease was transmitted, just as in all the earlier instances [22, 23].

In the eighteenth century, prayers for protection from the plague spread among the population. The chapel of Saint Roch was built in Subotica for that purpose [24]. For the same purpose, crosses can still be found on the roads in the region of Vojvodina. The fear spread among the general population primarily due to the sight of nodes on the body, followed by a 40–41°C fever. Similarly, the effects of the disease were noticed among the population in Serbia in the 19th century [25–27]. Sterile corridors and medical cordons were practiced in Serbia in the 1830s and 1840s, which was very significant to the Habsburg Monarchy. Dealing with the plague was in the jurisdiction of the Ministry of Internal Affairs and later the Ministry of Education. Close to the border with the Ottoman Empire, seven quarantine points were established. The two most significant quarantine points were in Supovac village near Aleksinac and Janko's Gorge (Serbian: *Jankova klisura*) along the river Blatašnica between Kruševac and Kuršumlija. The report from the Aleksinac quarantine showed the emergence of plague circles in Novi Pazar and Pešter hills. Quarantine in Radujevac (today's Ne-

gotin) showed an outbreak of plague in Silistrija, Bulgaria. The Ministry of Internal Affairs of Serbia reported that the Albanian riot at the southern border might have posed a threat to the further spread of the plague and that the southern medical corridors had to be strengthened [28]. It was also noted that the Romanian government had decided to set up quarantine facilities on the left bank of the Danube river alongside the Radujevac quarantine. In March 1840, an order was issued for all packages sent in large quantities from Turkey to Belgrade to be cleaned in quarantine and taxes charged. Austrian sources imply that there was no plague in the neighboring Turkish provinces, but the situation was quite different in more distant regions [29].

Plague outbreak in Srem and the consequences of the extent of the infection

In 1795, the plague broke out in Srem twice, first on the Military Frontier, where it was easily suppressed due to the sanitary measures whose implementation was overseen by the military authorities, and then, that same year in July, on the territory adjacent to the Military Frontier, where it spread quickly through the prosperous town of Irig and the surrounding villages. At first, the sanitary personnel did not believe it to be the plague. The first death cases were diagnosed with a severe infection caused by poor nutrition, mainly insufficiently baked bread made of recently harvested wheat or immature wheat grains. That was the opinion until the beginning of August when Doctor Andras Budai came to Irig and the village of Neradin and observed the symptoms of the disease and the changes it caused in people. He noticed that the patients suffered from fever, headaches, debility, weakness, and chills. This first phase of the illness was followed by drowsiness and absence of mind, whereby the patients would forget about hunger and thirst, at which time the first signs of derangement were manifested. Additional symptoms were nausea and the urge to vomit, followed by extreme pain. He noticed that between the second and fourth day, the patient began to suffer from painful swelling, differing in size, in the loin area, inner thighs, below the knee, armpit, and glands around the ears. He established that towards the end, *petechiae* of different sizes and colors emerged and that the dying "looked horrific", as well as that death occurred on the second or third day after the first symptoms, although more frequently on the sixth or seventh day. The symptoms and manifestations, as Doctor Budai described them, clearly confirmed that these were cases of plague [30].

Measures to prevent the disease from spreading were immediately taken: guards were placed around the parts of the town where the infected patients died or simply around the houses with reported death cases; a building was allocated for receiving patients, and a separate burial site was organized; an order was issued forbidding all burial rituals and enforcing compulsory burning of the deceased clothing; the villages and the surrounding crop fields were closed off; sanitary cordons were organized; quarantine facilities and guard posts were erected; disinfection measures were implemented in the infected areas where all the less valuable items were burned, and the more valuable washed, often by adding alcoholic beverages or vinegar, which were thought to have medicinal properties; the homes were disinfected by burning saltpeter, bran, and sulfur or by pouring vinegar on red-hot iron. To facilitate the operation of all organizations involved in preventing the spread of the epidemic, a special royal commissioner was appointed to deal with all the issues. That person was Baron Josef Pichler, and Doctor Franz van Schraud was sent to the infected areas [31].

As is the case with all the restrictions, the sanitary measures prescribed before and during the epidemic were only partially successful in limiting the spread of the plague. It soon became apparent that folk customs, especially those regarding burial rituals, presented a serious problem in the process of eradicating the epidemic. One of the customs – giving away the deceased belongings to relatives and friends after the funeral – contributed a great deal to the spread of the infection, which was thus passed on to healthy individuals. In the beginning, the plague also spread because the deceased were always bathed, which was another part of the burial ritual, and by doing so, the individuals who performed the bathing also became infected through direct contact. The spread of the infection created a state of chaos, which resulted in noncompliance with the sanitary measures and regulations issued for the exact purpose of preventing the spread of the disease from the already infected to the noninfected areas. That became so widespread that the only Serbian grammar school, located in Sremski Karlovci, was closed during the 1795/96 school year because both the students and teachers fled in fear of the epidemic [32]. The population resisted the order to isolate the ill in separate buildings while the patients were often left to fend for themselves, so they left their homes and died in the surrounding vineyards, forests, gardens, etc. Only after the bodies of the deceased were discovered were they buried on the spot where they had been found, most often in improperly dug shallow graves without notifying doctors or state authorities. Due to fear, people refused to participate in any work involving the ill, including the construction of hospitals. On the other hand, they were unwilling to give up the burial ceremonies, which included carrying an open casket with the deceased, whose body was kissed at least once during the prayer for the departed soul or during the wake, which sometimes lasted up to several days. The order stating that the deceased had to be buried within 48 hrs was not abided by, and so there were instances when the deceased were not buried for as long as eight days [33]. Similar measures had roots and good practices from 16th century England [34].

The mortality rate was the highest during field work season, at the time when the population intermingled the most. The epidemic in Irig peaked in October 1795, at least regarding the number of deaths. The crop fields of these villages bordered each other, and the villages themselves were not located far from one another. The people were interconnected as neighbors, friends, relatives, or simply through trade which enabled easier contact between the infected and noninfected individuals and the spread of the epidemic. In the second group of villages, the plague broke out in August

and took a great number of lives during the next three months. This group included Rivica and Jazak, two villages in the close vicinity of Irig, the town which suffered a catastrophic human loss. The third group represented villages to which the plague was transmitted from an already infected region, most often accidentally, as was the case with Veliki Radinci, Grgurevci, Bešenovo, Kamenica, Bešenovački Prnjavor, and the town of Vukovar. The plague did not spread to these villages until the end of October, excluding Vukovar; therefore, the greatest number of deaths occurred in November. In these cases, the number of deaths concerning the number of inhabitants is far less than in the previous two groups. However, since the number of those infected in the third group was significantly lower, the spread of the plague within them points more to carelessness than poor control [35].

The doctors turned to the Serbian Orthodox Church for help in their battle against the plague because they were aware that strict adherence to religious customs was one of the ways the infection was being spread. Knowing who might be able to help in such a situation, the doctors approached Archbishop Stefan Stratimirović of Karlovci (1790–1836), who wrote to the ministry and archpriests through his exarch, Stefan Avakumović, hoping that his advisories would help calm the state of chaos, which was the first step in eradicating the disease. In August, the exarch had already sent a letter forbidding priests from bringing the deceased into churches. Then he ordered that the deceased be taken to the cemeteries without their family or friends being present and with the assistance of individuals whose job was to perform burials. Finally, he ordered them not to allow the custom of kissing the deceased. He also asked the ministry to close all the churches immediately and hold services outdoors, but his orders and requests fell on deaf ears. The churches remained open, and public burials and the rituals that accompany them were continued as if there was no epidemic. While the high clergy was aware of all the benefits of these measures, the lower priesthood was not, least of all the congregation, and so, not only did they not abide by Avakumović's orders, but the churches were kept open all through 1795, and the burials organized with open caskets. By doing so, the priests not only disregarded the orders of their superiors but also aided in spreading the infection. Similar methods can be seen a few decades later in the region of Dalmatia [36].

After October 1795, the plague began to recede because this was the end of all field work, and there was no intermingling between the healthy and the infected population. Only then were all the measures that were meant to subdue the plague truly enforced, including isolation of both homes with infected individuals or death cases, as well as entire villages and regions. For this purpose, in addition to guards composed of the general population, the military was also distributed to all five posts, i.e., sanitary cordons. Another fact contributing to the reduction of the epidemic had to do with the erection of cordons, providing tighter closure, which additionally prevented communication between the infected and non-infected regions. Still, the most beneficial measure was isolating the infected in separate buildings. On the outskirts of most villages, buildings were erected during September and October where the patients were placed and thus isolated from the rest of the population. It soon became evident that this decision was a good one because although the number of deaths in quarantine was high, the number of new cases of infection was low, almost insignificant. Separating and sending the infected to treatment facilities and then doing the same with the rest of the population (e.g., all the citizens of Irig had to go through a six-week quarantine at the time when the plague had already been stopped, so the town was completely deserted for a time) contributed to slowing down the epidemic followed by its complete eradication. When the population abided by the sanitary measures, especially the measure stating that infected individuals or entire families were to be isolated from those who were not infected, the spread of the disease was easily stopped and quickly eradicated. There are two examples of such cases: the villages of Grgeteg and Bešenovo. In the first village, following the initial fear of the plague, the inhabitants accepted the advice of the doctors – they placed the infected individuals or families into quarantines and positioned guards around the village. As a result, almost half of the infected died in the period 1795–1796 before these measures were imposed. The rest of the infected died while in quarantine, with only a few individuals who did not abide by the measures and came into contact with the inhabitants of Neradin, where the plague was desolating the village at that same time. Bešenovo is the second example of how quick action, this time taken by the citizens themselves, resulted in the isolation and destruction of the plague. In this village, the inhabitants took it upon themselves to isolate the first family struck by the plague, and thus no one else in the village became ill.

They managed to stop the plague from spreading and then wiped it out altogether only after executing the sanitary measures – ceasing all burial rituals and customs, providing doctor supervision, and strictly forbidding traffic across cordon lines. In that way, they prevented the plague from spreading deeper into the region of the Habsburg Monarchy, which would have undoubtedly resulted in much higher mortality. The prevention of the plague outbreak continued in Serbia in the following decades, which had a massive impact on the health situation in the Habsburg Monarchy. Most of the dead in Serbia have been thoroughly investigated, especially during outbreaks of plague in 1831, 1836, and 1837. Medical doctor Carlo Nagy from the Austrian Zemun quarantine, helped Serbian doctors detect the disease [37]. In the summer of 1837, several cases of plague were detected in Jagodina, Paraćin, and Ražanj [38]. The Serbian medical service was determined to stop the disease. State Commissioner for Plague Control Avram Petronijević reported on September 1837 that 120 persons, the infected ones and those who were in contact with them, were held in prison [39]. Sanitary control was performed on the roads as well. Roads leading to infected settlements were closed with military guards. In infected settlements, the houses were cleaned, and the clothes of the infected were burned. Three houses in Valjevo were impossible to clean, so it was ordered to burn them [40]. After

all these medical measures, the number of those infected was declining, and the health situation improved.

According to the aforementioned, the following conclusions can be made: the first group of villages, where the inhabitants did not abide by the sanitary measures, was hit the hardest by the plague; in the second group of villages, where the inhabitants abided by the sanitary measures, partially or completely, the effects of the plague were much weaker. The greatest number of deaths occurred in the villages where the inhabitants did not abide by the sanitary measures from the very beginning, in other words, in the villages where burial rituals took precedence. A few decades later, in Serbia, medical measures and awareness of inhabitants were on a higher level which helped decrease the pressure on the southern borders of the Habsburg Monarchy.

Conclusion

The outbreak of the plague in the southern region of the Habsburg Monarchy in 1795 and 1796 provoked fear in the entire country and chaos among the inhabitants of Srem, where the epidemic had spread. It was demonstrated that in the first phase of the disease, the sanitary measures, including the overall health legislation, were powerless and that superstitions, religious rituals, and folk customs concerning burial practices had a much stronger influence. As a result, in some towns, with Irig being the largest, over half of the population succumbed to the disease. The spread of the epidemic was stopped and finally eradicated only after the military and secular authorities joined forces in the battle against the infection. Isolating the infected individuals, which violated the standard norms of behavior from the point of view of the patriarchal population, was the only way to stop the disease from spreading deeper into the Monarchy and causing catastrophic human losses, as in the case of the epidemic in the 1840s. Thus, more rigorous control was gradually imposed, and the towns in the southern region of the Monarchy, where the regulations were met with better reception and the folk customs repressed, had a significantly lower number of deaths. The better health situation was helped by excellent quarantine service in Serbia and medical cooperation between these two countries. Lethality, which was always high in all instances of plague epidemics, proved to be so in the southern region of the Habsburg Monarchy as well. Still, the epidemic was limited to towns along the border, and it did not have greater repercussions as far as the state was concerned, and this was the greatest benefit of the implemented sanitary measures.

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

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

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

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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.

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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.

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