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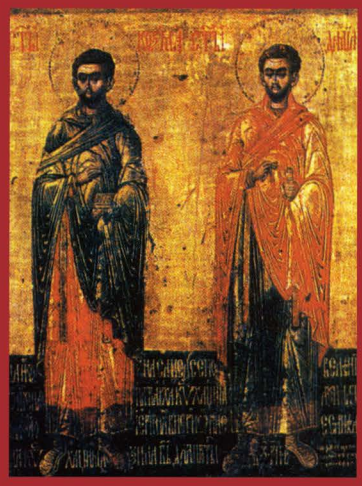

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Podružnice Srpskog lekarskog društva u Nišu

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and the Department of the Serbian Medical Society in Niš

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BLEEDING RISK FACTORS IN ACUTE CORONARY SYNDROME

Sonja Dakić[1,2], Goran Koraćević[1,2], Zoran Perišić[1,2], Tomislav Kostić[1,2], Svetlana Apostolović[1,2], Sonja Šalinger Martinović[1,2], Dušanka Kutlešić Kurtović[1], Danijela Đorđević Radojković[1,2], Nenad Božinović[1,2], Boris Đinđić[1,2], Dragana Drašković[3], Bojan Maričić[1], Jelena Perišić[1], Srđan Mijatović[4]

The modern treatment of patients with acute coronary syndrome (ACS) is justified and effective, but carries a higher risk of bleeding. The study aimed to determine the basic risk factors for bleeding and included 177 patients with ACS who were hospitalized at the Clinic for Crdiology Niš, in the period from May to September 2013. Based on the presence of bleeding, patients were first divided into patients with bleeding and patients without bleeding. Patients with bleeding were further divided into patients with significant bleeding and patients with less significant bleeding. The study took into account: demographic and anamnestic data, a form of ACS, basic laboratory tests and applied therapy for ACS. In the group of patients with bleeding, there were statistically significantly more non-smokers (χ^2 = 6.527, p = 0.038), patients with chronic kidney disease (CKD) (χ^2 = 4.192, p = 0.041) and patients with higher CRP values (p = 0.039). In the subgroup of patients with significant bleeding, there were a statistically significantly more frequent patients with CKD (36.4% vs. 6.5%, Fisher's tests: p = 0.007) and higher CRP values (z = 2.452, p = 0.014). In the subgroup of patients with less significant bleeding, the values of hemoglobin (t = 3,496, p = 0,003) were statistically significantly lower compared to other patients. It is interesting to note that the values of hemoglobin, creatinine clearance, and leukocyte count (as a parameter of inflammation) are variables of the PRECISE-DAPT scoring system that appeared in 2017, after our study. *Acta Medica Medianae 2023;62(2): 5-14.*

Key words: *acute coronary syndrome, bleeding, risk factors*

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Introduction

According to the current data from the World Health Organization (WHO), coronary heart disease (CHD) is the leading cause of death in the world and is responsible for 16% of all deaths. In Europe, CHD kills almost 1.8 million people a year, accounting for 20% of the total annual mortality rate. (1, 2)

Acute coronary syndrome (ACS), as the most severe form of CHD, is a life-threatening condition that requires urgent hospitalization and appropriate therapy. (3) According to the NHANES (National Health and Nutrition Examination Survey) from 2013 to 2016, the overall prevalence of myocardial infarction in the United States (USA) over the age of 20 was 3.0%, namely 4.0% for males and 2.3% for females. (4)

The treatment of patients with ACS is rapidly changing and improving with the advent of newer, more potent antithrombotic agents. A more aggressive antithrombotic therapeutic approach, aimed at reducing mortality, is justified and effective but carries a higher risk of bleeding, which is relatively common in routine clinical practice (5, 6, 7).

Until recently, bleeding in ACS (except for intracranial hemorrhage) was not given much importance, because it was considered that these complications were easily resolved and did not affect the prognosis. Today, based on numerous data, it is known that bleeding in ACS has a great

impact on mortality within 30 days and other adverse events. In the Global Registry of Acute Coronary Events (GRACE) registry, significant bleeding was associated with an increased risk of in-hospital mortality and proved to be an independent predictor of mortality (8). Minor bleeding usually does not require medical attention during the acute phase, however, there is evidence that it also has an impact on mortality and ischemic events, but to a lesser extent (8).

Accordingly, it is very important to define risk factors for bleeding, assess the individual risk for the patient, and based on that choose the appropriate therapeutic approach (invasive or conservative, stent type, as well as the appropriate choice of antithrombotic drugs), which would minimize the risk of bleeding and provide the best therapeutic efficacy (8, 9).

The aim of the research

The study aimed to determine the basic risk factors for bleeding and their relationship.

Respodents and methods

The study included 177 patients diagnosed with ACS who were hospitalized at the Clinic for Cardiology in Niš, in the period from May 15 to September 10, 2013. There were 120 men (67.8%) and 57 women (32.2%) in the study population. The average age of the examined population was 63.79 ± 12.30 years (min - 26, max - 90 years). The diagnosis of ACS included both patients with myocardial infarction (IM) and ST-segment elevation (STEMI) and those without ST-segment elevation (NSTEMI), and with unstable angina pectoris (UA). Based on the presence of bleeding during hospitalization, patients were first divided into two groups: patients who had bleeding and patients who did not have bleeding. Within the group of patients with bleeding, based on the ACUITY criteria (Acute Catheterization and Urgent Intervention Triage strategy) definition of bleeding, patients were further divided into patients with significant (major) bleeding and patients with less significant (minor) bleeding. The study took into account: demographic data of patients, body weight at admission and BMI, anamnestic data, a form of ACS (STEMI, NSTEMI, UA), values of systolic and diastolic blood pressure at admission, basic laboratory tests, and complete blood count, applied therapy for ACS.

Statistical data processing

The collected data were coded and entered into a specially formed database. Statistical processing was performed in the software package SPSS 16.0. The t-test, χ^2 test, or Fisher's test of exact probability (if the absolute frequency was less than 5) and Mann-Whitney's U test were used. The statistical hypothesis was tested at the level of significance for the risk of $\alpha = 0.05$, i.e. the difference between the samples is considered significant if $p < 0.05$. Logistic regression analysis was used to prove potential risk factors for bleeding.

Research results

The number of smokers and non-smokers was equal in the examined population (38.4%). No patient had a history of previous bleeding, and one patient had a history of previous thrombosis (0.6%). Five patients (2.8%) had a history of a previous ulcer. The prevalence of diabetes mellitus in the study population was 28.8%, and the prevalence of hypertension was 77.4%. Fifteen patients (8.5%) had chronic kidney disease (CKD) history.

In the study population, there were 23 patients (13.0%) with bleeding, of which 11 patients (6%) had significant bleeding, and 12 patients (7%) had less significant bleeding (Figure 1).

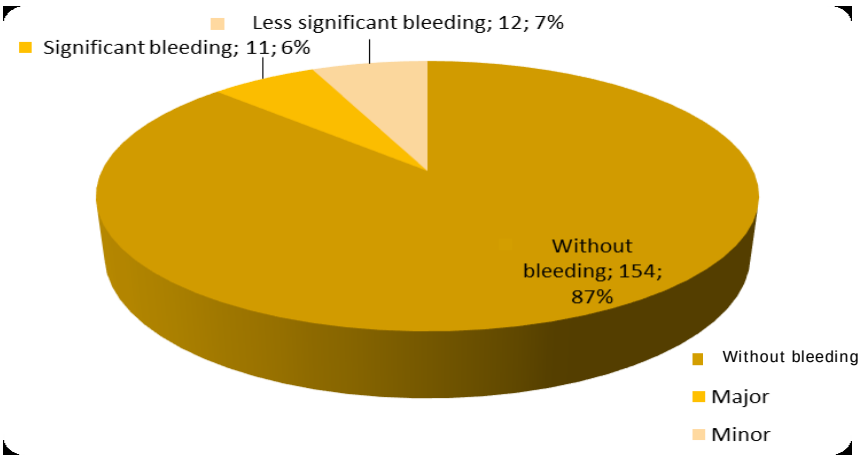

Figure 1. The prevalence of bleeding in the examined population

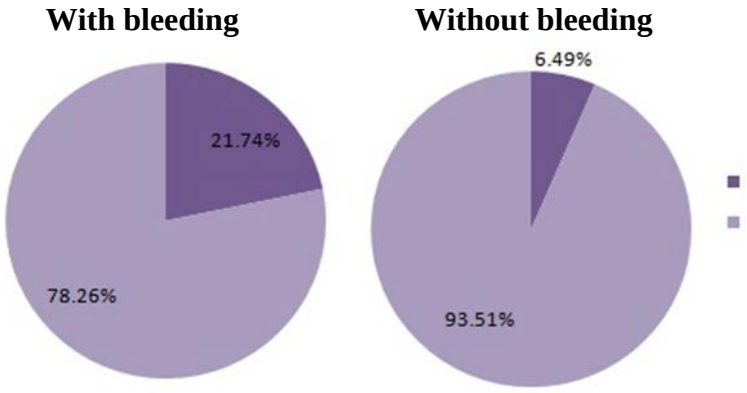

Figure 2. The presence of CKD history in relation to the occurrence of bleeding

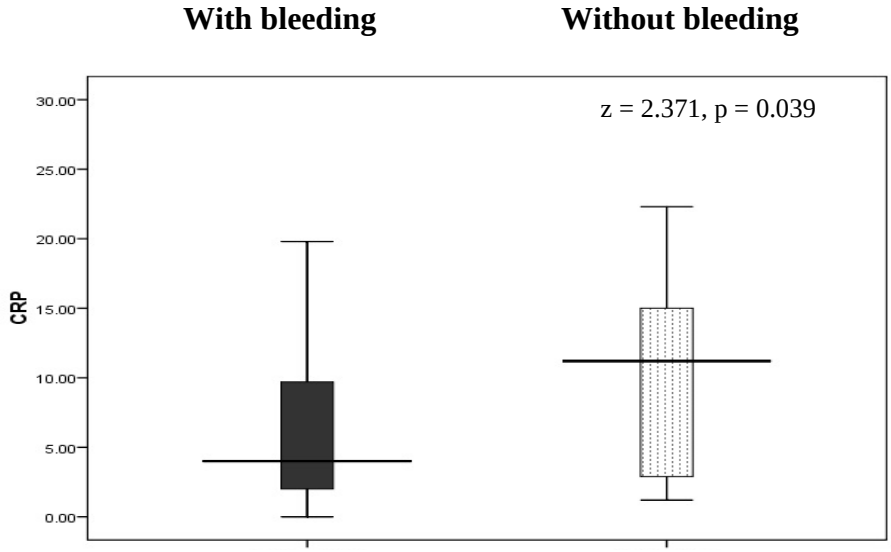

Figure 3. CRP values in relation to the occurrence of bleeding

In the group of patients with bleeding, there were statistically significantly more non-smokers (χ^2 = 6.527, p = 0.038). Further, the anamnesis of chronic kidney disease (CKD) was statistically significantly more frequent in patients with bleeding, compared to other patients (χ^2 = 4.192, p = 0.041) (Figure 2).

Likewise, in the subgroup of patients with significant bleeding, it was shown that patients with significant bleeding had a statistically significantly more frequent history of CKD compared to other patients (36.4% vs. 6.5%, Fisher's tests: p = 0.007). Comparing other demographic and clinical parameters concerning the occurrence of bleeding, no statistically significant difference was found.

It was determined that there was no statistically significant difference in the values of systolic and diastolic blood pressure, body weight, and laboratory tested parameters concerning the occurrence of bleeding, except for the value of C-reactive protein (CRP), which was statistically significantly higher in patients with bleeding compared to patients without bleeding (p = 0.039) (Figure 3).

Similarly, in the subgroup of patients with significant bleeding, CRP values (z = 2.452, p = 0.014) also proved to be statistically significantly higher compared to patients without bleeding. The values of other examined parameters did not differ statistically significantly in the occurrence of significant bleeding. In the univariate logistics model, CRP stood out as an independent risk factor (OR 1.014, p = 0.036). Non-smokers have a 5 times higher risk of bleeding than smokers. These statistically significant risk factors were then included in a multivariate logistic regression in which CRP was singled out as a statistically significant factor (OR 1.013, 95% CI 1,000-1.026, p = 0.047).

In the subgroup of patients with less significant bleeding, the values of hematocrit (t =

3.376, p = 0.004) and hemoglobin (t = 3.496, p = 0.003) were statistically significantly lower compared to patients without bleeding. Decreases in hemoglobin and hematocrit have been identified in the univariate statistical model as independent risk factors for the development of minor bleeding. These two risk factors did not prove statistically significant in the multivariate model. The values of other examined parameters did not differ statistically significantly compared to the occurrence of less significant bleeding.

It was shown that there is a statistically significant difference in the occurrence of bleeding in relation to certain forms of ACS (χ^2 = 4.12, p = 0.042). Patients with bleeding (87.0%) have statistically significantly more frequent STEMI compared to patients without bleeding (63.0%). In the bleeding group, one patient (4.3%) had newly developed atrial fibrillation (AF) and two patients had previously had AF. In the group of patients without bleeding, five patients (3.2%) had newly developed AF, and four had AF from before. No statistically significant difference was found in the frequency of newly developed AF (p = 0.572) and AF from earlier (p = 0.175) in relation to the occurrence of bleeding.

All patients from both groups received initial doses of aspirin and clopidogrel with gastroprotective therapy, at the first contact with the medical service. Patients received appropriate doses of anticoagulant therapy (unfractionated heparin or enoxaparin) according to the European guideline for ACS treatment. PCI was used in 16 patients with bleeding (69.6%) and 109 patients without bleeding (70.8%). It was determined that there is no statistically significant difference in the frequency of PCI, in relation to the occurrence of bleeding (p = 0.899).

In the subgroup of patients with less significant bleeding, it was shown that they were statistically significantly more likely to receive fibrinolysis and subsequent PCI during hospitalization compared to patients without bleeding (Fisher's test: p = 0.026).

Discussion

A review of the existing literature in order to find data related to the prevalence and risk factors for bleeding in ACS in our country did not provide the required data. The results of this research were viewed mainly in the light of data from large registers and studies.

The effect of smoking on bleeding

Smoking leads to numerous harmful effects that are manifested on both the cardiovascular system and the body as a whole. Endothelial dysfunction, dyslipidemia, elevated levels of inflammatory markers, and altered rheological properties of the blood are just some of the mechanisms that classify smokers as people at an increased risk for the occurrence and development of ischemic heart disease (10–14) .

By processing the data of a large number of studies and registers, it was shown that smokers with ACS had a higher risk of bleeding. Smoking, as a risk factor for bleeding, is also one of the variables of some scoring systems for assessing the risk of bleeding (15, 16). Smoking also affects certain drugs used in ACS. Thus, smoking affects the metabolism of clopidogrel by inducing cytochrome P450 (CYP) 12A, which converts clopidogrel to its active metabolites, and leads to a greater degree of inhibition of platelet activity (17). Some authors have shown that smokers on clopidogrel have a higher risk of bleeding, moderate to severe, but some claim that such patients are not at increased risk (10, 18).

In the examined population, it was shown that bleeding was significantly more frequent among non-smokers. In addition to numerous publications where smoking is a risk factor for bleeding, there is also a certain number of publications, in which bleeding is more common in non-smokers, as it is in our study. By analyzing the basic characteristics of patients in the GUSTO I study, which included 41,021 patients with ACS, Berkowitz et al. showed that bleeding was more common among non-smokers (19). Al-Mallah and his colleagues examined the predictors of GIT bleeding and showed that it was more common among non-smokers (20). Examining the effects of clopidogrel in ACS in smokers and non-smokers, Sibbald et al. showed that there was no greater reduction in adverse events in smokers compared to non-smokers and that major bleeding was less common among smokers (18). There are several mechanisms, which could explain the more frequent bleeding among non-smokers. Namely, due to the effects that smoking has on the cardiovascular system, primarily the occurrence of endothelial dysfunction, dyslipidemia, accelerated atherosclerosis, and increased thrombogenicity, smokers get coronary heart disease earlier than non-smokers do. Because they are younger, they usually have fewer comorbidities. Non-smokers who get coronary heart disease are in most cases older (which is the case in the study population) and have more comorbidities (DM, CKD, HTA) that were involved in the development of coronary heart disease, and which are also risk factors for bleeding. In addition, as they get older, they are at a higher risk of bleeding, because the patient's age is an independent predictor of bleeding (8). Given that thrombogenic factors are increased in smokers, then they could be expected to have a lower risk of bleeding than non-smokers. In addition, they may be less likely to have anemia that is a risk factor for bleeding because they often have polycythemia as a compensatory mechanism. Toss and associates noticed that when dalteparin is used in patients with ACS, at a dose of 120 IU/kg twice daily, there is a higher anti-Xa activity in women and non-smokers, which

increases the tendency to bleeding. Perhaps this could also be one of the mechanisms that would explain the higher frequency of bleeding among non-smokers (21).

CRP as a marker of inflammation and a risk factor for bleeding

Elevated CRP levels, detected in the first days after myocardial infarction, may reflect an elevated systemic inflammatory response, induced by myocardial necrosis, cytokine release, and activation of the inflammatory cascade (22). In the last fifteen years, several publications have been published, where CRP is an independent predictor of a bad outcome in patients with NSTEMI. Tsakiris and associates have shown that high CRP at admission is a predictor of short-term and long-term adverse events (22). Inflammation certainly has an impact on the occurrence of adverse events in ACS, including bleeding, with some authors arguing that an elevated number of white blood cells is a more powerful predictor of adverse events, including bleeding, than CRP (23). The fact that Mehran, Nikolsky, and associates in the scoring system for assessing the risk of bleeding have included elevated leukocytes as a marker of inflammation certainly shows that the inflammatory condition is a risk factor for the occurrence of bleeding in ACS (24).

In our population, it was shown that the values of CRP were significantly higher in patients with bleeding compared to those without bleeding, as well as in the group with significant bleeding compared to patients who did not bleed. This association of CRP values with bleeding did not exist in patients with less significant bleeding. In the univariate logistic model, CRP was singled out as an independent risk factor (OR 1.014, p = 0.036), and in the multivariate logistic regression, CRP was singled out as a statistically significant factor (OR 1.013, 95% CI 1,000-1.026, p = 0.047). The mechanisms by which inflammation affects the occurrence of bleeding have not been fully elucidated. One of the possible mechanisms might be damage to the integrity of endothelial cells in the process of inflammation, damage to the vascular barrier, and an increase in vascular permeability. In addition, elevated CRP values are a marker of an inflammatory condition, and inflammation may be associated with many other conditions and comorbidities, which increases the susceptibility to bleeding. Since it has been shown that there is a correlation between CRP values and infarct severity, patients with higher CRP values, and thus more severe infarction, are more likely to have more comorbidities, which affects the severity of the disease. This primarily refers to patients with long-term DM, CKD, who have more severe heart attacks due to more extensive changes in blood vessels, and it is well known that both DM and CKD are proven risk factors for bleeding. Inflammatory markers are also elevated in anemic patients, and they are also at higher risk of bleeding. In addition, elevated CRP values are associated with exacerbations of many chronic inflammatory diseases that are more common in the elderly, and age is an independent predictor of bleeding.

Chronic kidney disease as a risk factor for bleeding

It is a known fact that the frequency of adverse events is higher in patients with varying degrees of CKD, and this relationship exists throughout the ACS spectrum (25). El-Menyar et al. have shown that CKD is associated with multiple increases in mortality in patients with ACS, namely: three times higher mortality in mild CKD, ten times higher in moderate, and as much as eighteen times higher mortality in severe CKD (25). Analysis of the GRACE registry data showed that CKD is an independent risk factor for bleeding and that the risk of significant intrahospital bleeding in renal patients increases by approximately 50% (8). Similarly, Manoukian and associates confirmed that CKD is an independent risk factor for ACS bleeding (26). The parameters of renal function are also part of the scoring systems for assessing the risk of bleeding. Thus, the values of serum creatinine are part of the scoring system of Mehran and Nikolsky, and the values of creatinine clearance are part of the CRUSADE system (24, 27). Creatinine clearance is one of the five variables included in the PRECISE-DAPT (Predicting Bleeding Complications In patients Undergoing Stent Implantation and Subsequent Dual Antiplatelet Therapy) scoring system, which emerged after our study (28).

It is of great importance to know renal function in patients with ACS because, in patients with impaired renal function, who are at risk for bleeding, by taking appropriate preventive measures, this risk can be minimized. Choosing a radial approach over the femoral can also reduce the risk of bleeding (29).

Our study also showed that patients with a history of CKD were 5.5 times more likely to have significant bleeding than patients with normal renal function. This correlation was not confirmed in patients with less significant bleeding. Several mechanisms can be used to explain the increased risk of bleeding in patients with renal dysfunction. Circulating uremic toxins are partly responsible for platelet dysfunction, affecting both platelet activation and aggregation, as well as the interaction between platelets and the arterial wall (30). In patients with ACS and CKD, due to delayed elimination of antithrombotic drugs, which are partially or completely excreted by the kidneys, there is a high possibility of overdose with these drugs if the dose is not adjusted to renal function and therefore a higher risk of bleeding. Patients with CKD very often have associated conditions that are a cause or consequence of CKD, and which are also risk factors for bleeding, such as hypertension,

diabetes, anemia. These are often elderly patients, which in turn increases the risk of bleeding.

Anemia and the risk of bleeding

Anemia is associated with an increased risk of adverse events, which exist throughout the spectrum of coronary heart disease, including stable angina pectoris, ACS, and even in patients undergoing elective PCI (29). Low hemoglobin levels at admission are an independent predictor of mortality in acute coronary syndrome, and also a risk factor for the development of heart failure and bleeding (31). The effect of anemia on the increased risk of bleeding could be explained by several mechanisms. One of the possible explanations would be the effect of hyperdynamic circulation, as a compensatory mechanism on anemia, on the more vulnerable endothelium due to hypoxia. In addition, other risk factors for bleeding are very often present in anemic patients, which further increases the risk of bleeding.

Namely, anemic patients are mostly elderly people, in whom anemia is caused by inadequate nutrition, poor absorption of essential ingredients for normal erythropoiesis, very often occult bleeding, and it is well known that age is an independent predictor of bleeding. Anemia is more common in females, and women are also at higher risk of bleeding than men.

Very often, anemia is associated with renal dysfunction, which through several mechanisms affects the occurrence of anemia, primarily due to reduced erythropoiesis, due to lack of erythropoietin, and the presence of uremic toxins that disrupt erythropoiesis. The presence of low hemoglobin on admission may reflect the presence of undetected hemorrhagic diathesis, which leads to an increased risk of bleeding after anticoagulant and antiplatelet therapy. Anemia in chronic diseases is not uncommon, therefore, low hemoglobin on admission can be a companion of an inflammatory condition that affects the occurrence of anemia through several mechanisms, primarily negatively affecting erythropoiesis and reducing the absorption of iron from the gastrointestinal tract. Inflammation is associated with an increased risk of bleeding. The inflammatory response is an integral part of the reaction to tissue damage in myocardial infarction and can persist for several weeks. Increased cytokine production may adversely affect erythropoiesis and impair intestinal iron absorption (32).

In the study population, the values of hemoglobin and hematocrit at admission were significantly lower in patients with less significant bleeding, compared to patients who did not bleed. This correlation was not found in patients with significant bleeding, which suggests that anemia is certainly a risk factor for bleeding, with less significant bleeding being mostly puncture site bleeding in our study, which could be easily controlled, so did not become significant bleeding. Another explanation could be that some other risk factors for bleeding were probably crucial in patients with significant bleeding. Decreases in hemoglobin and hematocrit were performed in a univariate statistical model as independent risk factors for the development of minor bleeding. These two risk factors did not prove statistically significant in the multivariate model.

The association of ACS with bleeding

In the examined population, bleeding was more common among patients with STEMI form of ACS, compared to NSTEMI and APNS. These results are consistent with the results of most authors. Based on data from the ACUITY and HORIZONS AMI studies, Mehran, Nikolsky, and associates formed an evaluation system for assessing the risk of bleeding, where one of the factors to be evaluated is the presence of STEMI at admission (24). Since patients with STEMI have a fibrin-rich thrombus, they also receive reperfusion therapy, in terms of PPCI or fibrinolytics. As a result, more patients receive several antithrombotic drugs simultaneously during the first few days of myocardial infarction (33). There is also an increased risk of bleeding with invasive procedures. Patients over 80 undergoing PCI are especially at high risk of bleeding (34). The incidence of bleeding in percutaneous interventions is lowest in elective PCI and highest in primary PCI (34). Based on that, it could be concluded that STEMI patients who undergo PPCI are at higher risk of bleeding compared to NSTEMI and APNS.

The concomitant use of PCI and fibrinolysis and the risk of bleeding

Reperfusion therapy, including PPCI and thrombolysis, is a standard in the treatment of patients with STEMI. Based on the results of a meta-analysis, which included data from 23 clinical studies, PPCI proved to be much more effective than thrombolytic therapy in terms of reducing mortality, reinfarction, and stroke. However, in several patients, thrombolytic therapy remains the treatment of choice, due to the fact that PPCI is not available in many centers, where there is no possibility of rapid transport of the patient to the catheterization room (35, 36). In such cases, after thrombolysis, the patient should be transported to a PCI center as soon as possible. In case of failed thrombolysis, or if there is evidence of re-occlusion or reinfarction with recurrent ST-segment elevation, the patient should be referred to emergency rescue PCI (5). However, in addition to the benefits, rescue PCI is associated with a higher incidence of non-fatal bleeding, compared to PPCI (37). This is mainly the case of an increased incidence of minor bleeding, especially at the puncture site (38, 39).

It is not so rare that after a failed attempt to open the infarct artery, the patient develops fibrinolysis. In our study, it was shown that in

patients who received both fibrinolytic and PCI, less significant bleeding was more frequent, which is in accordance with the data from the literature. Among patients with significant bleeding, there was no significant association between concomitant PCI and thrombolysis and bleeding, which is again consistent with the literature data.

Conclusion

Bleeding in patients with ACS was more common among non-smokers.

Patients with a history of CKD, as well as patients with elevated CRP values, were also statistically significantly more likely to bleed. This association also existed in a subgroup of patients with significant bleeding.

Patients with less significant bleeding were significantly more likely to have anemia on admission compared to patients without bleeding, as well as those with significant bleeding.

Bleeding was shown to be more common in patients with STEMI ACS, and concomitant use of fibrinolysis and PCI was associated with a more frequent occurrence of less significant bleeding, mainly at the puncture site.

In the univariate logistic model, CRP stood out as an independent risk factor, and in the multivariate logistic regression, CRP stood out as a statistically significant factor.

It is interesting to note that the values of hemoglobin, creatinine clearance, and leukocyte count (as a parameter of inflammation) are variables of the PRECISE-DAPT scoring system that appeared in 2017, after our study.

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FAKTORI RIZIKA ZA NASTANAK KRVARENJA U AKUTNOM KORONARNOM SINDROMU

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Savremeni terapijski pristup kod bolesnika sa akutnim koronarnim sindromom (AKS) jeste opravdan i efikasan, ali nosi sa sobom veći rizik od nastanka krvarenja. Cilj istraživanja, koje je obuhvatilo 177 bolesnika sa AKS, hospitalizovanih na Klinici za kardiovaskularne bolesti u Nišu u periodu od marta do septembra 2013. godine, bio je utvrditi osnovne faktore rizika za nastanak krvarenja. Na osnovu prisustva krvarenja, bolesnici su najpre bili podeljeni na bolesnike sa krvarenjem i bolesnike bez krvarenja. Bolesnici sa krvarenjem dalje su podeljeni na bolesnike sa značajnim krvarenjem i bolesnike sa manje značajnim krvarenjem. Prilikom istraživanja u obzir su uzeti sledeći podaci: demografski i anamnestički podaci, oblik AKS-a, osnovne laboratorijske analize, kao i primenjena terapija za AKS. U grupi bolesnika sa krvarenjem bilo je statistički značajno više nepušača (χ^2 = 6,527, p = 0,038), bolesnika sa hroničnom insuficijencijom bubrega (HBI) (χ^2 = 4,192, p = 0,041) i bolesnika sa povišenim CRP vrednostima (p = 0,039). U podgrupi bolesnika sa značajnim krvarenjem bilo je statistički značajno više bolesnika sa HBI (36,4% prema 6,5%; Fišer test: p = 0,007) i povišenim CRP vrednostima (z = 2,452, p = 0,014). U podgrupi bolesnika sa manje značajnim krvarenjima, vrednosti hemoglobina bile su statistički značajno niže u odnosu na ostale bolesnike (t = 3,496, p = 0,003). Interesantno je pomenuti da su vrednosti hemoglobina, klirens kreatinina i broj leukocita (kao marker inflamacije) sastavne komponetnte PRECISE DAPT skoring sistema, koji se pojavio 2017. godine, nakon našeg istraživanja. *Acta Medica Medianae 2023;62(2):5-14.*

Ključne reči: *akutni koronarni sindrom, krvarenje, faktori rizika*

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GENERALIZED PAIN HYPERSENSITIVITY IN FIBROMYALGIA PATIENTS

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Chronic widespread pain is one of the leading symptoms of fibromyalgia. Signs of generalized hyperalgesia can often be observed in these patients. However, it is not clear if the pain hypersensitivity is present for different painful stimuli. Therefore, the aim of this study was to determine if there were differences in pressure pain threshold - PPT, heat pain threshold - HPT and cold pressure threshold - CPT between fibromyalgia patients and healthy subjects. The present cross-sectional study included 45 subjects (average age 54.60 ± 7.96 years, 88.9% females), of whom 23 (51.1%) were diagnosed with fibromyalgia, while 22 (48%) were the healthy control group in whom PPT, HPT and CPT were measured on the forearm and on the paraspinal musculature of the lumbosacral region of the spinal column. Fibromyalgia patients had a significantly lower PPT compared to the group of healthy subjects: 26.13N/cm2 vs. 53.54N/cm2, (Z=-4.439, p<0.001); HPT 39.70 °C vs. 44.85°C, (Z=-3.871, p<0.001); CPT 20.51°C vs. 12.51°C, (Z=-2.612, p=0.009). In the area of the paraspinal musculature, PPT was 37.01 N/cm2 vs. 75.77 N/cm2, (Z=-4.178, p<0.001); HPT - 38.18°C vs. 44.13°C (Z=-3.758, p<0.001); CPT - 21.52°C vs. 11.16°C (Z=-2.737, p=0.006). Fibromyalgia patients demonstrated generalized hyperalgesia for all tested modalities (pressure, heat and cold). *Acta Medica Medianae 2023;62(2): 15-22.*

Key words: *pain threshold, fibromyalgia, hyperalgesia*

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Introduction

Fibromyalgia (FM) represents a chronic painful condition characterized by chronic widespread pain, sleep problems, chronic fatigue, headache, depression, functionality and cognitive impairments (1–3). FM affects around 5% of population, most of which are women usually around 30−35 years old (4). The diagnosis of fibromyalgia is made exclusively by history data based on the criteria of the American College of Rheumatology (ACR) from 2016 (5). We still do not understand the mechanisms of pain origin in FM completely. However, it is assumed that the altered process of pain signal processing is probably the main cause of pain in FM. It most often occurs in genetically predisposed individuals, and the triggers of fibromyalgia can be diverse (4, 6, 7). Dysfunction of endogenous modulation system in the CNS plays an important role in generalized pain sensitivity seen in patients with FM. This increased responsiveness of nociceptive neurons in CNS is defined by the IASP as central sensitization (CS) (8). CS is characterized by allodynia (pain to touch and other stimuli that normally do not cause pain) and hyperalgesia (excessive response to minimally painful stimuli). Although mechanisms that lead to CS are not fully understood, increased activity of neurons of the posterior horns of the spinal cord and activation of N-methyl-D-aspartate (NMDA) receptors, central afferent pain enhancement and reduction of central descending inhibition (facilitation) play an important role (4). Various methods of examining central sensitization have been developed (9–11), of which quantitative sensory testing (QST) stands out (12). QST is a method that provides insight into pain thresholds and differentiation of local versus generalized and peripheral versus central neural mechanisms (13, 14). QST results depend on the age, gender (15–20) and body composition (21, 22). The prevailing opinion is that repeated exposure to painful stimuli can increase the pain threshold. On the contrary, it has been shown in numerous studies that chronic pain can lead to the

opposite effect and decrease the pain threshold (23–25). Signs of generalized hyperalgesia can often be observed in fibromyalgia patients. However, it is not clear if pain hypersensitivity is present for different painful stimuli (26–28). Therefore, the aim of this study was to determine if there were differences in pressure pain threshold - PPT, heat pain threshold - HPT and cold pressure threshold - CPT between fibromyalgia patients and healthy subjects.

Material and methods

The research was designed as a cross sectional study. It was conducted at the Medical Rehabilitation Clinic of the University Clinical Centre of Vojvodina, after obtaining the consent of the Ethics Committee of the University Clinical Centre of Vojvodina. Participation in the study was completely voluntary and all participants signed informed consent.

Pain thresholds for pressure, heat and cold between healthy subjects and patients with previously diagnosed FM were compared, using locally applied pressure, heat and cold on both the forearm and the lower back.

The sample consisted of a total of 45 respondents (average age 54.60 ± 7.96 years). The sample consisted of two groups, a control group of healthy subjects (n=22, average age 51.8 ± 5.20, females 81.8%) and an experimental group of FM patients (n=23, average age 57.3 ± 9.23, females 95.7%).

The criteria for inclusion (in the experimental group) in the research were: age over 18 years, diagnosed with FM based on the ACR 2016 criteria (5).

The criteria for inclusion (in the control group) in the research were: age over 18 years, absence of any chronic pain condition or current acute pain.

Exclusion criteria were: age under 18 years and incomplete data, presence of cancer, rheumatic inflammatory disorders, diabetes mellitus, multiple sclerosis, polyneuropathy, Parkinson's disease, and patients who were using neuroleptics, strong opioids, or benzodiazepines on a regular basis.

All involved subjects were tested according to the protocol for pain thresholds examination previously developed by Knezevic et al. and Kovacevic et al. (19, 29).

In the present study, data on pressure pain thresholds (PPT), heat pain thresholds (HPT) and cold pain thresholds (CPT) were examined. A digital algometer (Wagner Instruments, FDX-50), with a rubber tip with a diameter of 1 cm, was used to test PPT. With this algometer, PPT was examined in 2 places: 1) on the paravertebral musculature of the lumbar segment (2−3 cm lateral from the processus spinosus of the L3 vertebra), 2) on the opposite forearm (proximal part of the body of the extensor carpi radialis longus). We gradually increased the pressure with the algometer at a speed of about 5 N/s, and the subject said "stop" as soon as the feeling of pressure turns into a feeling of burning, stinging, stabbing or pain, and the value in N/cm2 was recorded. At each place, 3 measurements were taken, with an interval of 10 s, and their mean value was taken as the final value. The left/right side was tested randomly.

HPT and CPT were examined using a device (Pathway Pain and Sensory Evaluation System, Medoc Ltd, Ramat Yishai, Israel) using an ATS (Advanced Thermal Stimulation) thermode measuring 30x30mm. HPT and CPT were examined at 2 sites: 1) on the paravertebral musculature of the lumbar segment (2−3 cm lateral to the spinous process of the L1 vertebra) and 2) on the proximal volar side of the opposite forearm (C8 dermatome). The left/right side was tested randomly. During HPT/CPT testing, the initial temperature of 32°C increased/decreased at a rate of 0.7°C/s. The subject pressed the stop button as soon as the hot/cold sensation changed to burning, stinging, stabbing or pain. At that moment, the temperature decreased/increased to the initial temperature, at a rate of 7°C/s. Four stimuli for hot and then four stimuli for cold were performed with an intermediate interval of 10 s, and the final value of HPT and CPT were taken as the mean values of the last three measurements.

Pain thresholds (first PPT, then HPT and CPT) on the forearm were first tested while the subject was lying on his back. Then the subject turned on his stomach and first the PPT, then the HPT and the CPT on the lumbar segment were examined at previously defined places.

Data analysis

SPSS 20.0 software package was used for data entry and processing. For the purposes of analysis and description of the structure of the sample according to relevant variables, frequency and percentage displays were used to show the representation of a certain category or response. Descriptive statistics methods were used to determine measures of central tendency (arithmetic mean), measures of variability (standard deviation) and extreme values (minimum and maximum) of observed numerical characteristics. The limited sample size and the broken normality of the distribution of certain parameters allowed the use of non-parametric methods. Within the comparative statistics method, the Mann-Whitney U-test was used for differences between two independent samples. In the applied tests, the limit values of the probability of risk are at the significance level of 95% ($p<0.05$).

Results

There was no difference in age, gender and BMI between healthy controls and FM patients (for more details see Table 1).

Pain threshold for pressure, heat and cold in the forearm area was significantly lower in subjects with FM compared to the control group. Given that the samples were small, non-parametric statistics (Mann-Whitney Test) were used for comparison between groups. PPT, HPT and CPT in the lower back region were significantly lower in subjects with FM compared to controls (Table 2).

Table 1. Demographic characteristics of participants

	Healthy controls (n=22)	Fibromyalgia patients (n=23)	Total (n=45)	$t/Z/x^2$	p
Age (mean ± SD) (years)	51.8 ± 5.20	57.3 ± 9.23	56.6 ± 7.96	Z=-1.910	p= 0.056
Gender (female, %)	18 (81.8%)	22 (95.7%)	40 (88.9%)	X^2= 2.179	p=0.187
BMI[1] (mean ± SD) (kg)	24.37 ± 3.03	26.02 ± 3.50	25.21 ± 3.35	t= -1.638	p= 0.100

[1]*BMI = Body Mass Index (kg/m2)*

Table 2. Differences in pain thresholds

	Fibromyalgia patients	Healthy controls	Z	p
Forearm				
PPT (mean ± SD) ($N/cm^{2)}$	26.13 ± 11.26	53.54 ± 26.13	Z= - 4.439	P<0.001
HPT (mean ± SD) (°C)	39.70 ± 4.14	44.85 ± 2.77	Z= - 3.871	P<0.001
CPT (mean ± SD) (°C)	20.51 ± 8.33	12.51 ± 10.20	Z= -2.612	P=0.009
Lower back				
PPT (mean ± SD) ($N/cm^{2)}$	37.01 ± 22.79	75.77 ± 34.84	Z= -4.178	P<0.001
HPT (mean ± SD) (°C)	38.18 ± 4.49	44.13 ± 3.96	Z= -3.758	P<0.001
CPT (mean ± SD) (°C)	21.52 ± 10.40	11.17 ± 11.16	Z= - 2.737	P=0.006

Discussion

Sensitivity to pain is an individual characteristic of each person and is conditioned by various factors, from ethnic, psychophysical, psychological, genetic, demographic and social factors (30–35).

The results of this research show that the pain threshold for pressure in the area of both the forearm and the low back was significantly lower in subjects with FM compared to the control group. Pressure pain threshold represents minimum pressure quantity applied to particular body site able to produce sensation of pain (36). Transduction of mechanical noxious stimuli includes mechanical stimulation of unmyelinated C-MH fibers (37) and myelinated A-HTM fibers (38). Majority of the studies found decrease of PPT in fibromyalgia patients (26, 39–43).

Similar to the PPT we found that the pain threshold for heat and cold in the forearm and low

back area was significantly lower in subjects with FM compared to the control group. Majority of studies showed similar result to our study (43–48), while certain authors did not find differences in heat and cold pain thresholds between FM patients and healthy controls (27, 28). A possible reason for the discrepancy in the results of the studies is that in the study by Klauenberg et al., respondents were allowed to continue taking pharmacological therapy (in addition to coanalgesics and other medications, more than a third of the respondents were on NSAIDs and/or opioids), while the use of NSAIDs, narcoleptics, opioids and benzodiazepines in our study was a criterion for exclusion from the research. Nerve fibers implicated in heat and cold transmission are A-delta mechano-heat fibers and C-polymodal fibers (49). Transduction of heat sensation involves nociceptive afferent fibers which leads to release of glutamate and peptides in the dorsal horn of spinal cord (50). These substances play role in excitation of second order sensory neurons and projection neurons in spinothalamic tract (STT) (51, 52).

Recently, more and more attention has been paid to the process of neuroinflammation in FM. Neuroinflammation is thought to be responsible for many painful conditions possibly affecting process of CS (53). It implies the process of glial activation and activation of astrocytes with the release of an abundance of proinflammatory factors (54). As part of FM, there are changes in the serum and cerebrospinal fluid concentration of certain neurotransmitters, and it is assumed that they lead to increased sensitivity to pain because these transmitters are associated with the processes of pain transmission and modulation. It is known that the level of substance P is elevated in patients suffering from FM (55). It was also concluded that other biochemical changes occurred in FM, primarily reduced concentrations of serotonin, dopamine and noradrenaline metabolites (antinociceptive neurotransmitters) and high concentrations of substance P and nerve growth factor (pronociceptive, excitatory neurotransmitters) (56). The assumption that the dysfunction of the inhibitory descending system is to a certain extent responsible for the widespread pain in FM corresponds to the reduced concentration of the neurotransmitters serotonin and noradrenaline that occurs in this disease because these transmitters are responsible for the normal modulation of pain (descending pathways of the brain to the neurons of the posterior horns of the spinal cord) (57). In addition to the pain syndrome, changes in serotonergic transmission can explain the occurrence of other symptoms in FM, such as sleep and mood disorders (58). CS may explain the state of central hyperexcitability of the nociceptive system and the consequent reduced pain threshold in patients with FM (47). By shifting the modulation towards facilitation, the sensory "inflow" increases, resulting in sensitization, which is clinically manifested by a diffuse painful condition in the absence of peripheral disease (59). This supports the results we obtained. Other studies suggest reduced habituation to pain (60) and CS as mechanisms (61). In their study, Giesecke et al. (62) found hyperalgesia in FM patients and chronic back pain patients compared to healthy controls when experimental pain was applied to a neutral site, i.e., a location where FM patients had no pain. In addition to the neurotransmitter and neurosensory explanations, we can also explain the occurrence of greater sensitivity to pain in FM by the existence of pronounced persistent sympathetic (catecholamine) hyperactivity with a paradoxical hypoactive response to stress. The state of impaired autonomic regulation affects the manifestation of physical and psychological symptoms of FM. The mentioned mechanisms can, to a lesser or greater extent, increase the sensitivity to pain in an individual. All above mentioned mechanisms indicate the presence of non-selective CS in patients with FM. This CS leads to hyperalgesia to all modalities which we have proved in the present study.

Conclusion

Pain thresholds for pressure, heat and cold are significantly lower in subjects with FM, both in the forearm and lower back regions indicating the presence of generalized pain hypersensitivity to different modalities in patients with FM.

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GENERALIZOVANA PREOSETLJIVOST NA BOL KOD OBOLELIH OD FIBROMIJALGIJE

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Hronični široko rasprostranjeni bol je jedan od glavnih karakteristika fibromijalgije. Kod ovih pacijenata često se uočavaju znaci generalizovane hiperalgezije. Međutim, nije jasno da li je prisutna povećana osetljivost na bol za različite bolne stimuluse. Stoga, cilj ove studije bio je da se ustanovi da li postoje razlike u pragu bola za pritisak (engl. pressure pain threshold – PPT), pragu bola za toplo (engl. Heat pain threshold – HPT) i pragu bola za hladno (engl. Cold pain threshold – CPT) između pacijenata sa fibromijalgijom i zdravih ispitanika. Ova studija preseka uključila je 45 ispitanika (prosečne starosti 54.60 ± 7.96 godina, 88,9% žena), od kojih 23 (51.1%) boluje od fibromijalgije, dok su 22 (48%) bili kontrolna grupa zdravih ispitanika kod kojih PPT, HPT i CPT mereni na podlaktici i na paraspinalnoj muskulaturi lumbosakralne regije kičmenog stuba. Pacijenti oboleli od fibromijalgije imaju značajno niži prag bola na pritisak u poređenju sa grupom zdravih ispitanika: 26.13N/cm2 naspram 53.54N/cm2, (Z=-4.439, p<0.001); HPT 39.70°C naspram 44.85 °C, (Z=-3.871, p<0.001); CPT 20.51°C naspram 12.51°C, (Z=-2.612, p=0.009). U regiji paraspinalne musculature PPT je bio 37.01 N/cm2 naspram 75.77 N/cm2, (Z=-4.178, p<0.001); HPT - 38.18°C naspram 44.13°C (Z=-3.758, p<0.001); CPT - 21.52°C naspram 11.16°C (Z=-2.737, p=0.006). Kod pacijenata obolelih od fibromijalgije prisutna je generalizovana hiperalgezija za sve testirane modalitete (pritisak, toplotu i hladnoću). *Acta Medica Medianae 2023;62(2):15-22.*

Ključne reči: *prag bola; fibromijalgija; hiperalgezija*

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EFFECTS OF THE ETHANOLIC LEAF EXTRACT OF *ANETHUM GRAVEOLENS* L. ON CONTRACTILE ACTIVITY OF ISOLATED RAT ILEUM AND TRACHEA

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Dill (*Anethum graveolens* L., Apiaceae) has been used for centuries as a spice, as well as a remedy for gastrointestinal problems in traditional medicine. The aim of this study was to evaluate the effects of the ethanolic leaf extract of *Anethum graveolens* L. (AGEE) on the smooth muscle contractile activity of rat isolated ileal and tracheal strips. AGEE was obtained by using ultrasonic extraction from air-dried and powdered leaves of cultivated Anethum graveolens L. This study examined the effects of AGE on the spontaneous, KCl (80 mM), Acetylcholine and $CaCl_2$-induced smooth muscle contraction of isolated rat ileum, as well as on the contractile activity of isolated rat trachea induced by Carbachol and KCl (80 mM). Results showed that AGEE produced significant ($p < 0.01$) concentration-dependent relaxation of spontaneous and induced contractions of ileal smooth muscle. Addition of AGEE significantly ($p < 0.01$) reduced in a dose dependent manner the contractile effects of the carbachol and KCl on the isolated rat trachea. Our findings indicate that AGEE decreased contractile response of rat ileal and tracheal smooth muscle. *Acta Medica Medianae 2023;62(2): 23-30.*

Key words: *dill, Anethum graveolens L., extract, rat, ileum, trachea.*

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Introduction

Herbal medicine research is very extensive because medicinal plants represent a great therapeutic potential for the prevention and treatment of wide range of diseases. Dill (Anethum graveolens L.) is a plant of the Apiaceae family, and has a long history of use, both as a medicinal and aromatic plant (1). In Ayurvedic medicine, preparations of A. graveolens are well known as carminative, stomachic and diuretic agents (2). Dill has been used in herbal medicine for the treatment of mental disorders, convulsions, asthma, thyroid diseases (3, 4), to regulate the menstrual cycle, to reduce labour pain, to increase milk production in lactating women, to prevent colic in babies and alleviate pain effects (5, 6). Numerous studies demonstrated that dill might also have hepatoprotective, anti-inflammatory, antithrombotic, analgesic, apoptogenic, antioxidant, antibacterial, antifungal, larvicidal, anti-hyperlipidaemic, antidiabetic, and cerebroprotective properties (7-12).

It is known that the fruit hydroalcocholic extract of A. graveolens inhibited the contractions of the isolated rat ileum (13). Also, Jafarzade et al. (14) showed relaxant effect of seed hydroalcoholic extract of A. graveolens on isolated rat trachea. However, studies on effects of the A. graveolens leaf extract in the isolated rat intestine and trachea were not found in the available literature.

The Aim

The aim of our study was to investigate the effects of the ethanolic leaf extract of A. graveolens on the spontaneous and induced contractions of isolated rat ileal and tracheal strips.

Material and methods

Drugs and Reagents

The following drugs were used: Acetylcholine chloride (Sigma Chemical Co-USA), Carbamoylcholine chloride (Carbachol, Sigma Chemical Co-USA), Verapamil (Sigma Chemical Co-USA), Atropine sulphate (Sigma Chemical Co-USA) and Papaverine hydrochloride (Merck, Darmstadt, Germany).

Preparation of the Extract

The plant material (leaves) was collected in the surrounding area of Niš, and a voucher specimen for A. graveolens was deposited in the Herbarium of the Faculty of Science and Mathematics, Department of Biology and Ecology, University of Niš, Serbia, with the accession number 16420. The leaves were then air-dried, pulverized and extracted in an ultrasonic bath with 96% ethanol. The extract was concentrated in a rotary evaporator. The obtained dry residues were dissolved in the distilled water.

Animals

Male Wistar albino rats were kept under standard laboratory conditions. All the experimental procedures with the animals were in compliance with the European Council Directive of September 22nd, Directive 2010/63/EU and were approved by the Animal Ethical Committee of the Faculty of Medicine in Niš (No: 01-206-7).

Tissue Preparation

Overnight fasted animals were sacrificed by cervical dislocation. The trachea was dissected and immediately placed in a Krebs solution, whereas the ileum was placed in a Tyrode's solution. The change of tissue segments contractility was recorded using a TSZ-04-E Spell Iso system (Experimetria Ltd., Budapest, Hungary).

Ileum

After a dissection of the abdominal cavity, the ileum segments were isolated and cleaned off mesenteries. Ileal strips were mounted in an organ bath containing a Tyrode's solution (37ºC, aerated with carbogen). The rat ileum was cumulatively treated with AGEE (0.02-6 mg/mL). Papaverine (0.1-30 μM) was used as a control substance. The relaxant effect was expressed as a percentage change of the basal tone compared with baseline values. The potential anticholinergic activity was examined by the cumulative addition of acetylcholine (5-1500 nM), and then concentration response curves were obtained in the presence of AGEE (6 mg/mL), or atropine (140 nM). To evaluate the possible calcium channel blocking activity of AG, the effects of AGEE or verapamil on the contractions of the rat ileum induced by KCl (80 mM) or $CaCl_2$ (0.01-3 mM) were recorded. In the calcium-induced contractions of the isolated rat ileum, a calcium-free Tyrode's solution was used, as described earlier (15, 16). The relaxation of the isolated ileum, which was precontracted with acetylcholine or calcium ions, was calculated as a percent of the agonists-induced contractions.

Trachea

The trachea was cleaned of connective tissue and tracheal rings containing 2–3 cartilage were prepared. Tracheal strips were mounted by inserting two stainless steel hooks in the lumen and placed in to organ baths containing oxygenated Krebs solution at 37 °C. The tracheal smooth muscle were contracted by KCl (80 mM) or carbachol (1 μM), and then AGEE was added. Verapamil was tested on KCl (80 mM) and atropine on carbachol-induced contractions (17, 18).

Statistical analysis

All results were expressed as mean±standard deviation (SD) of six determinations. A statistical significance of differences between two means was performed using the Student`s t-test. A probability value of $P < 0.05$ was considered to be significant. The half maximal effective concentration (EC50), the concentration which elicited 50% of maximal response, was established by a regression analysis. The data was analyzed using the SPSS statistical software package (v.20.0; SPSS, Chicago, IL, USA).

Results

AGEE in cumulative concentrations (0.02-6 mg/mL) produced a significant and concentration-dependent relaxation of rat ileum spontaneous contractions, with the EC50 values of 5.76±3.28 mg/mL, respectively (Table 1). Papaverine (0.1-30 μM) decreased the spontaneous rat ileum contractions with EC50 value of 2.01±0.01 μM (Figure 1).

The high concentration of K+ induced a tonic contraction in the rat ileum smooth muscle. AGEE caused a significant and concentration-dependent relaxation of the KCl induced contractions in the isolated rat ileum (EC50 values 6.38±0.49 mg/mL) (Table 1). The EC50 value of verapamil was 0.36±0.028 μM (Figure 2).

AGEE dose dependently reduced the rat ileum contractions stimulated by acetylcholine. The EC50 of acetylcholine in the presence of AGEE (6 mg/ml, 489.02±27.25 nM) was significantly higher than the EC50 of acetylcholine alone (0.54±0.08 nM; $P < 0.001$) (Table 2). The value

of EC50 in the presence of atropine was 445.32±28.57 mM (Figure 3).

AGEE inhibited the $CaCl_2$ induced contractions in a Ca2+-free medium. The EC50 values of calcium ions alone (0.005±0.0009 mM) were significantly increased in the presence of AGEE (6 mg/ml, EC50 = 0.94±0.01 mM, P < 0.001) (Table 2). The EC50 value for calcium ions was affected by verapamil (8.67±0.61 mM) (Figure 4).

AGEE caused a significant and concentration-dependent relaxant effect on the KCl and carbachol induced precontractions of the isolated rat tracheal rings, with EC50 values of 6.24±0.49 mg/mL and 10.88±0.97 mg/mL, respectively (Figure 5). Verapamil produced inhibition of KCl-induced contractions with EC50 value of 0.22±0.01 μM. Atropine, as a positive control, abolished carbachol-induced contractions (EC50 value was 0.004±0.0003 μM) (Table 3).

Table 1. Half maximal effective concentration (EC50) values of the Anethum graveolens L. ethanolic leaf extract (AGEE), as well as of papaverine and verapamil on spontaneous and KCl-induced contractions in isolated rat ileum

Drug	EC50 values for spontaneous contractions	EC50 values for KCl-induced contractions
AGEE	5.76±3.28 mg/mL	6.38±0.49 mg/mL
Papaverine/Verapamil	2.01±0.01 μM	0.36±0.028 μM

*Results are expressed as mean ± SD (n=6).

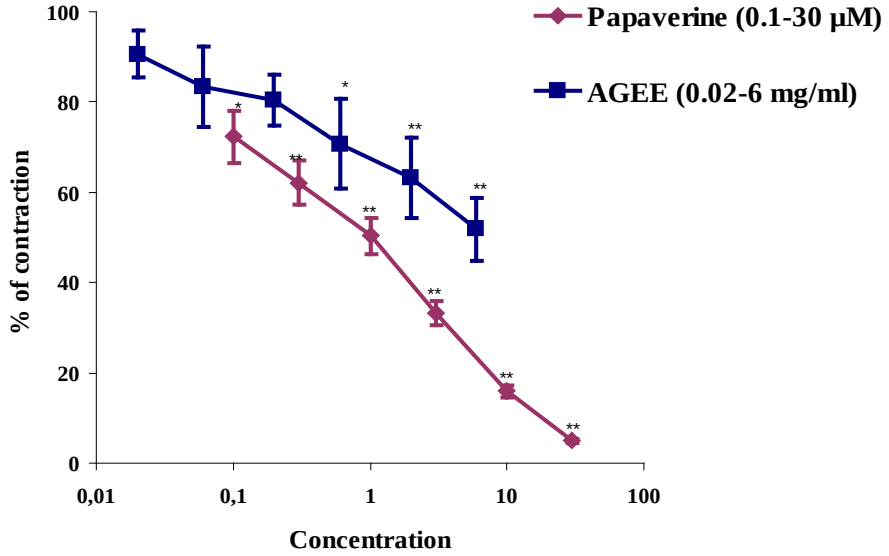

Figure 1. Relaxant effect of the Anethum graveolens L. ethanolic leaf extract (AGEE) and papaverine on spontaneous contractions in isolated rat ileum. Each data point represents the mean ± SD of the percentage values with respect to the spontaneous contractions in Tyrode solution (control). *p < 0.05, **p < 0.01 versus Tyrode (n=6)

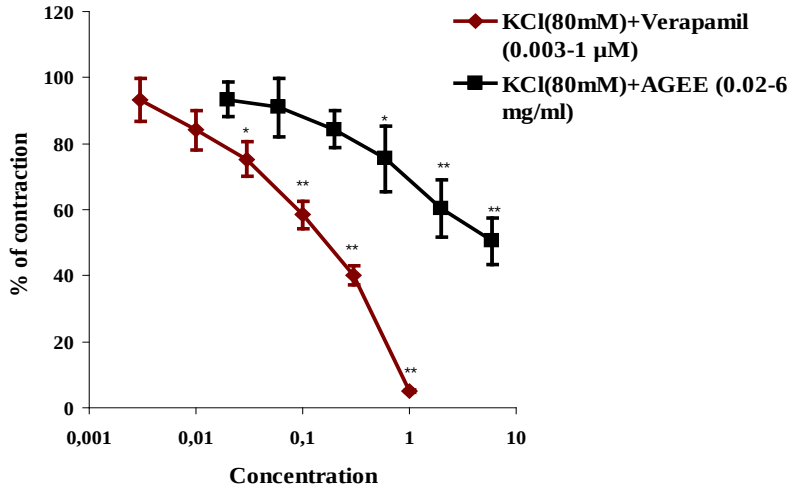

Figure 2. Relaxant effects of the Anethum graveolens L. ethanolic leaf extract (AGEE) and verapamil on ileum contraction induced by KCl (80 mM). Each data point represents the mean values in percent of maximal response±SD. *p < 0.05, **p < 0.01 versus control (n=6)

Table 2. Half maximal effective concentration (EC50) values of the Anethum graveolens L. ethanolic leaf extract (AGEE), as well as of atropine and verapamil on acetylcholine and $CaCl_2$-induced contractions in isolated rat ileum

Drug	EC_{50} values for Acetylcholine -induced contractions	EC_{50} values for $CaCl_2$-induced contractions
Control	0.54±0.08 nM	0.005±0.0009 mM
AGEE	489.02±27.25 nM**	0.94 ± 0.006 mM**
Atropine/Verapamil	445.317±28.57 mM	8.67±0.61 mM

*Results are expressed as mean ± SD (n=6). ** P < 0.01 significantly different when compared with control group.

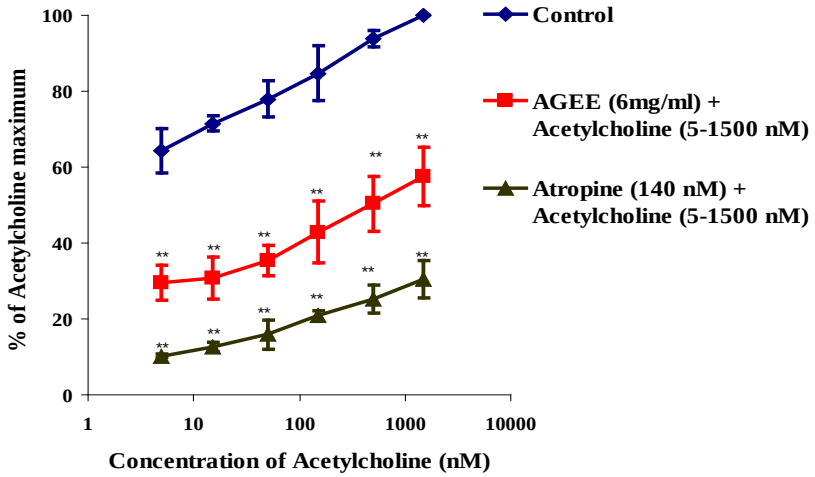

Figure 3. Relaxant effects of the Anethum graveolens L. ethanolic leaf extract (AGEE) and atropine on ileum contraction induced by acetylcholine. Each data point represents the mean values in percent of maximal response±SD. *p < 0.05, **p < 0.01 versus control (n=6)

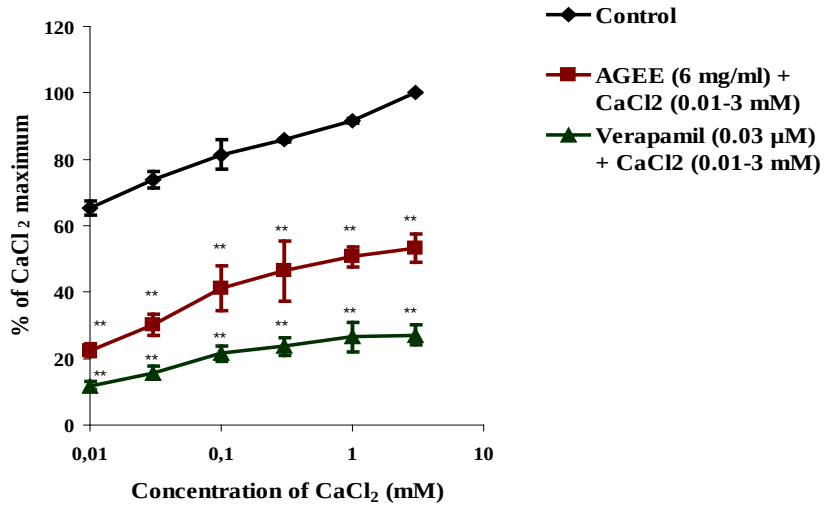

Figure 4. Relaxant effects of the Anethum graveolens L. ethanolic leaf extract (AGEE) and verapamil on contractions of the isolated rat ileum induced by $CaCl_2$. Each data point represents the mean values in percent of maximal response±SD. **p < 0.01 versus control (n=6)

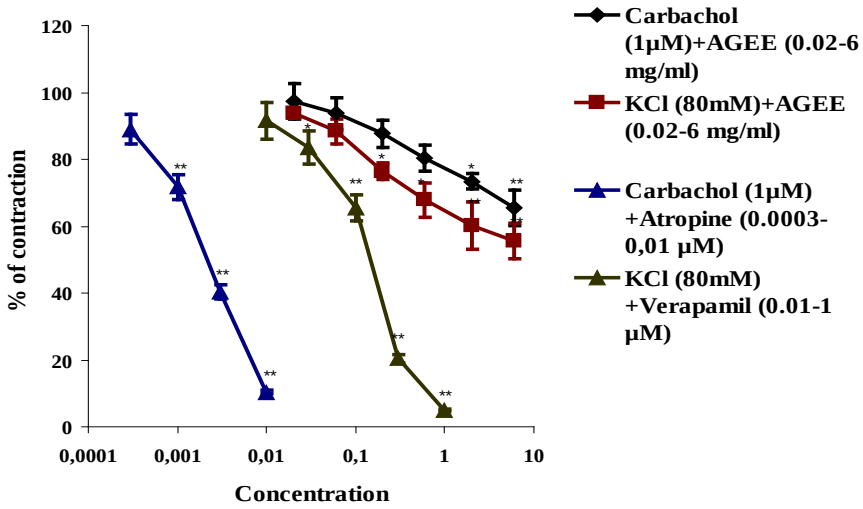

Figure 5. Relaxant effect of the Anethum graveolens L. ethanolic leaf extract (AGEE), verapamil and atropine on contractions of the isolated rat trachea induced by KCl and carbachol. Each data point represents the mean values in percent of maximal response±SD. *p < 0.05, **p < 0.01 versus control (n=6)

Table 3. Half maximal effective concentration (EC50) values of the Anethum graveolens L. ethanolic leaf extract (AGEE), as well of verapamil and atropine on KCl and carbachol-induced contractions in isolated rat trachea

Drug	EC_{50} values for KCl-induced contractions	EC_{50} values for Carbachol-induced contractions
AGEE	6.24 ± 0.49 mg/mL	10.88 ± 0.97 mg/mL
Verapamil/Atropine	0.22 ± 0.01 µM	0.004 ± 0.0003 µM

*Results are expressed as mean ± SD (n=6).

Discussion

The results of this study showed that AGEE induced a significant relaxant effects on the isolated rat ileum and rat trachea contractions. AGEE inhibited spontaneous and acetylcholine, KCl and $CaCl_2$-induced contractility of the rat ileum strips and relaxed the carbachol and KCl-contracted trachea. The muscle relaxant responses of AGEE were similar to a smooth muscle relaxant such as papaverine, atropine, a nonselective muscarinic antagonist and verapamil, a calcium channel blocker.

The neuroendocrine mediator, acetylcholine, is an important regulator of gastrointestinal motility (19). AGEE induced a significant depression of the cumulative concentration response curve for acetylcholine in the isolated rat ileum. Atropine, an antagonist of the muscarinic receptors, inhibited acetylcholine induced contractions. The interactions of acetylcholine with muscarinic receptors in the intestinal smooth muscle induce a G protein-mediated signal transduction that activates phospholipase C, resulting in an increase of intracellular calcium, depolarization and the contractions of the smooth muscle (20). Muscarinic acetylcholine receptors present on the smooth muscle cells are potential therapeutic targets for intestinal motility disorders. AGEE significantly inhibited acetylcholine stimulated rat ileum contractions, indicating a possible anticholinergic effect. These results may explain the traditional use AG for digestive system disorders. A relaxant potential of the methanol extract (21) and the hydroalcoholic fruit extract of dill (13) was also reported.

Many studies have shown that the activation of actin and myosin contractile filaments of the smooth muscle occurs due to increased sarcoplasmic free calcium (15, 16, 22, 23). The rise in the concentration of calcium could result from the influx of calcium through voltage-dependent ion channels and the secondary release from intracellular stores. A possible relaxant mechanism of the AGEE may be mediated through an inhibition of calcium channel pathways. High concentration of KCl induced both a depolarization and a tonic contraction of ileum smooth muscle trough the activation of voltage operated L-type calcium channels (24). In this study, AGEE inhibited ileal contractions induced by KCl. The spasmolytic effect was similar but milder compared to those of the calcium antagonist verapamil. Also, this study found that the pretreatment of the isolated rat ileum with the AGEE reduced $CaCl_2$ induced smooth muscle contractions in the calcium-free medium, similar to the verapamil calcium channel blocker. The obtained results indicate that the spasmolytic effect of the AGEE is possibly mediated through the calcium influx reduction from the extracellular fluid.

Our results on the spasmolytic effect of AGEE are confirmed by a study with clinical application of the dill powder of the crude herb in patients with irritable bowel syndrome (25). Inhibited acid secretion and lesions in the rats stomach in pretreated rats with dill extracts has also been noted in the literature (26).

When looking at the use of AG to treat respiratory diseases, AGEE was studied in isolated rat trachea to reveal its underlying mechanisms of relaxant activity. AGEE caused a significant relaxation of tracheal smooth muscle contractions induced with KCl and carbachol. As expected, verapamil relaxed the tracheal contractions induced with KCl. Atropine, a muscarinic receptor antagonist also relaxed the carbachol-induced contractions. These results suggested that the relaxant effect of AGEE on isolated rat trachea might be related to combined reduction of calcium influx and the inactivation of muscarinic receptors. The obtained results of bronchodilatory activity of AGEE are in agreement with the already reported study that found the relaxant activity of dill hydroalcoholic extract (14).

Conclusion

The present results showed that AGEE significantly decreased contractions of the isolated rat ileum and rat trachea. AGEE inhibited spontaneous and acetylcholine, KCl and $CaCl_2$-induced contractility of the rat ileum and relaxed the carbachol and KCl-contracted trachea. The data we obtained suggested the possibly of an anticholinergic and calcium channel-blocking AGEE activity and may, at least partially, account for the traditional use of A. graveolens for stomach and respiratory disorders.

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UTICAJI ETANOLNOG EKSTRAKTA LISTA *ANETHUM GRAVEOLENS* L. NA KONTRAKTILNU AKTIVNOST IZOLOVANOG ILEUMA I TRAHEJE PACOVA

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Mirođija (*Anethum graveolens* L., Apiaceae) se vekovima koristi kao začin, ali i kao lekovito sredstvo za gastrointestinalne probleme u tradicionalnoj medicini. Cilj ove studije jeste da proceni efekte etanolnog ekstrakta lista *Anethum graveolens* L. (AGEE) na kontraktilnu aktivnost izolovanog ileuma, kao i traheje pacova. AGEE je dobijen ultrazvučnom ekstrakcijom iz osušenih i usitnjenih listova kultivisane mirođije. Ova studija pratila je efekte AGEE na spontane, KCl (80 mM), acetilholinom i $CaCl_2$ indukovane kontrakcije izolovanog ileuma pacova, kao i kontraktilnu aktivnost izolovane traheje pacova indukovane karbaholom i KCl (80 mM). Rezultati pokazuju da AGEE indukuje statističi značajnu ($p < 0,01$) i kontrakciono zavisnu relaksaciju spontanih i indukovanih kontrakcija izolovanog ileuma. Takođe, AGEE statističi značajno ($p < 0,01$) umanjuje, proporcionalno primenjenoj koncentraciji, kontraktilne efekte karbahola i KCl na izolovanu traheju pacova. Naši rezultati pokazuju da AGE smanjuje kontraktilnost glatke muskulature izolovanog ileuma i traheje pacova. *Acta Medica Medianae 2023;62(2):23-30.*

Ključne reči: *mirođija, Anethum graveolens (L.), ekstrakt, pacov, ileum, traheja*

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EPIDEMIOLOGICAL CHARACTERISTICS OF GIARDIASIS IN THE AREA OF BELGRADE DURING THE PERIOD FROM 2007 TO 2020

Sonja Giljača[1], Slavica Maris[1], Milutin Mrvaljević[2], Zorica Mrvaljević[3]

The aim of this paper was to analyze the epidemiological characteristics of Giardiasis around Belgrade from 2007 to 2020. A descriptive epidemiological study was applied. In the observed period, 265 new cases of giardiasis were registered in Belgrade, the average raw incidence was 18.92/100,000. Men (56%) were more likely to develop giardiasis than women (44%). Highest age-specific incidence rate was at the age of 2 years (10.3/100,000). The largest number of patients was registered in municipality Savski venac 106 (40%). Seasonal distribution indicates that the largest number of patients was registered in September and October. The epidemiological situation in Belgrade in the observed period shows a declining tendency of the number of patients and the incidence rate, which is most likely the result of improved hygienic and sanitary living conditions. *Acta Medica Medianae 2023;62(2): 31-37.*

Key words: *giardiasis, protozoa, diarrhea, hygiene*

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Introduction

Giardiasis is an infection mainly of the upper part of the small intestine caused by the flagellar protozoan Giardia Lamblia. Although in about 50% of cases the infection is asymptomatic, it can be accompanied by various intestinal symptoms, such as chronic diarrhea, steatorrhea, abdominal cramps, bloating, frequent soft and light fatty stools, fatigue, and weight loss. Fat absorption or liposoluble vitamins may occur. Extraintestinal invasion does not usually occur, but reactive arthritis can occur, and severe lambliasis can damage the duodenal and jejunal mucosa (1, 2). Giardia Lamblia protozoan infection is also associated with the development of nervous bowel syndrome and chronic fatigue (3).

Confirmed cases of giardiasis are defined as cases that meet the clinical description and criteria for laboratory confirmation (4). Laboratory diagnosis of Giardia mainly made by microscopic identification of cysts or trophozoites in stool samples, but several immunological tests and molecular methods are available for the diagnosis of giardiasis (5).

According to the latest data from WHO G: Lamblia is the third most common cause of diarrheal diseases worldwide with over 300 million cases per year, preceded only by rotavirus, Cryptosporidium parvum and hominis in the most vulnerable target group of children under five (3). The infection rate in asymptomatic children is 8−30% in developing countries and 1−8% in industrialized regions (5). The prevalence of Giardiasis in different regions ranges from 2−3% in industrialized regions to 30% in low-income and developing countries. Since 2004, Giardia has been classified as a „neglected disease initiative" by the WHO and is directly linked to poverty and poor drinking water quality (3).

Reservoir is human, possibly beavers and other wild and domestic animals. The infection is transmitted from person to person, cysts from the feces of the infected are transmitted through the hands to the mouth, especially in institutions and Children Care Facilities, which is probably the main way of spreading.

Localized epidemics can occur by ingesting cysts through fecal contaminated water, and much less frequently through fecal contaminated food. Sources of infection are unfiltered water from streams and lakes, which are accessible to fecal water contamination (1).

Infection is often self-healing, lasting an average of 3 to 25 days. People with AIDS can

have a much more severe and long-lasting infection. The period of infection can often last for months. Giardiasis is treated with antibiotics; the drug of choice is Metronidazole. The key preventive measures of giardiasis are: 1) education of family, staff and members of institutions, especially adults in children's institutions, on the implementation of personal hygiene and the need to wash hands before starting work with food, before meals and after using toilets; 2) filtration of city water sources that are exposed to human or animal feces contamination; 3) protection of water sources from fecal contamination; 4) hygienic method of fecal disposition (1).

The aim of this paper was to analyze the epidemiological characteristics of giardiasis around Belgrade during the period from 2007 to 2020.

Material and Methods

A descriptive epidemiological study was applied. For the analysis of epidemiological characteristics of lambliasis around Belgrade, data were collected from: reports of infectious diseases, surveys of patients, medical documentation, results of epidemiological and laboratory tests, annual reports on the movement of infectious diseases around Belgrade. The data of average raw incidence of giardiasis in the Republic of Serbia during the observed period were taken from the annual reports of infectious diseases in the Republic of Serbia.

As we used published official data, the permission of the Ethics Committee was not required.

Link:
https://www.batut.org.rs/download/izvestaji/Godisnji%20izvestaj%20o%20zaraznim%20bolestima%202019.pdf

Link:
https://www.zdravlje.org.rs/index.php/izvestaji/centar-za-kontrolu-i-prevenciju-bolesti

Statistical analysis

Proportions, raw and age-specific incidence rates were used in the data analysis. To calculate the incidence rates, the number of new cases of giardiasis for the observed year was used as a counter, and the number of Belgrade residents according to the 2011 census data was used as a denominator. Statistical data processing was done using the Microsoft Office Excel 2007 program.

Results

During the period from 2007 to 2020, 265 new cases of giardiasis were registered in Belgrade, and the average raw incidence was 18.92/100,000. The highest number of new cases (32) and the highest raw incidence rate (2.00/100,000) of giardiasis were registered in 2007, and the lowest number of patients (4) and the lowest raw incidence rate (0.24/100,000) were registered in 2020. The highest age-specific incidence rate of giardiasis was recorded at the age of 2 years (10.3/100,000), and the lowest at the oldest age (60 to 69 and 70 and more years) (0.1/100,000) (Table 1). The distribution in relation to gender indicates that 117 females became ill, which makes 44% and 148 males, that is 56% of all patients. The proportion of affected women to the number of men is 1:1.3, from which it can be concluded that in the observed period, men were more likely to get sick than women (Figure 1).

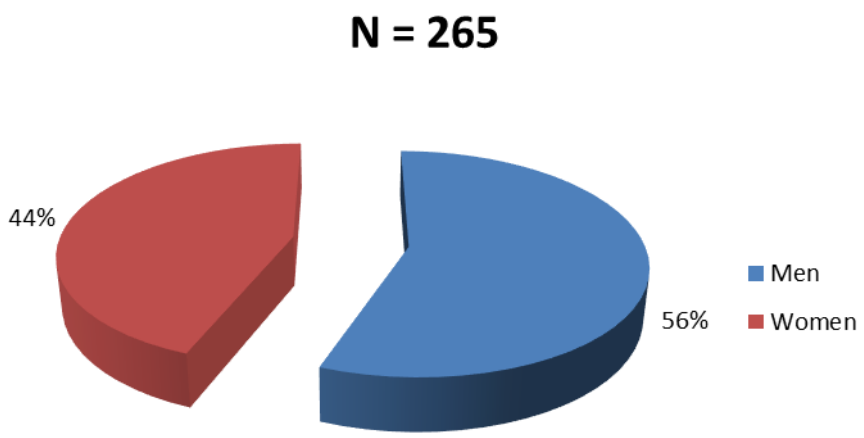

Figure 1. Distribution of new cases of giardiasis by gender, Belgrade, 2007–2020

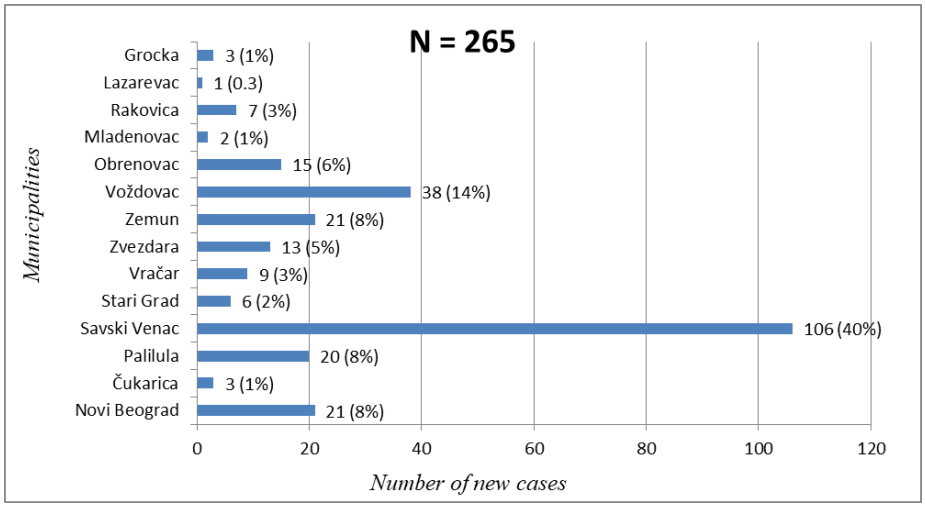

Figure 2. Distribution of new cases of giardiasis by municipalities, Belgrade, 2007–2020

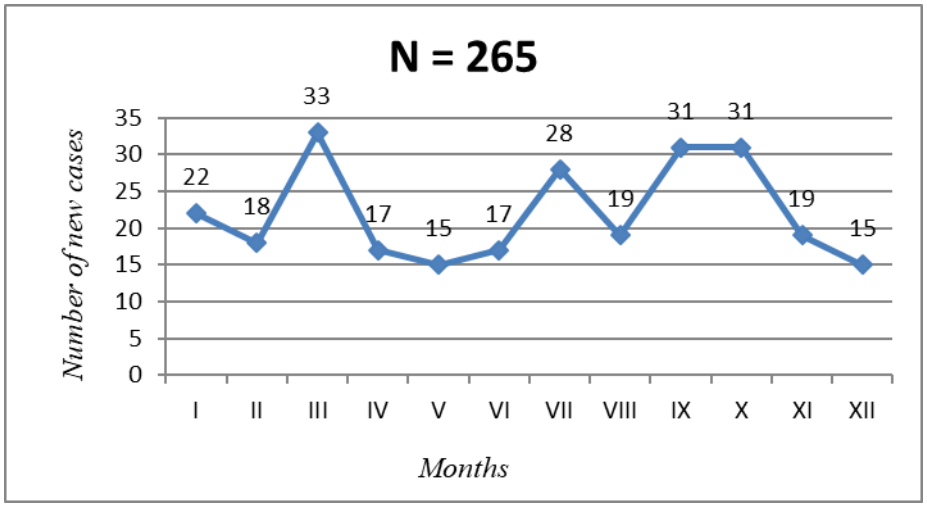

Figure 3. Distribution of new cases of giardiasis by months, Belgrade, 2007–2020

The highest number of patients was registered in the municipalities of Savski venac 106 (40%) and Voždovac 38 (14%), and the lowest in the municipalities of Lazarevac 1 (0.3%), Mladenovac 2 (1%), Grocka 3 (1%) and Čukarica 3 (1%). There were no registered cases of giardiasis in other Belgrade municipalities (Figure 2). Giardiasis is registered throughout the year with seasonal increases in summer and early autumn. The largest number of patients was registered in September and October. In that period, 62 patients (23.39%) became ill, and the lowest number of patients, 15 (5.6%), was registered in May and December (Figure 3).

Discussion

According to the results of our study, in the period from 2007 to 2020, 265 new cases of Giardiasis were registered in Belgrade, and the average raw incidence rate of giardiasis during the observed period ranged from 2.0/100,000 to 0.24/100,000 inhabitants (6). In Serbia, in the period from 2011 to 2017, the average raw incidence rate was from 1.77/100,000 to 1.29/100,000 inhabitants (7, 8). In the same period, in the Europe Union (EU), the average raw incidence of giardiasis ranged from 5.49/100,000 to 5.5/100,000, and in the United States (US) from 6.4/100,000 to 6.0/100,000 (9–12). In Belgrade, the number of people suffering from giardiasis was 1.3 times higher among men than women. As in our study, in EU countries, in 2016, the number of giardiasis patients was 1.3 times higher in men than in women (13). Observing the incidence of giardiasis by age groups in Belgrade, it was noticed that the age-specific rate of giardiasis was highest in the age group of 2 years (10.3/100,000),

Table 1. *Number of new cases and age-specific incidence rates (per 100,000) for giardiasis, Belgrade, 2007–2020*

(Age groups) (Year)	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2007-2020
<1 *No (Rate*)*	0	0	1(5.8)	0	0	0	0	0	0	0	1(5.8)	0	0	0	2(0.8)
1 *No (Rate*)*	0	1(5.8)	0	0	2(11.6)	1(5.8)	0	0	3(17.4)	1(5.8)	0	0	1(5.8)	0	9(3.7)
2 *No (Rate*)*	1(6.0)	0	1(6.0)	0	1(6.0)	0	1(6.0)	4(24.2)	3(18.1)	6(36.3)	4(24.2)	2(12.1)	0	0	24(10.3)
3 *No (Rate*)*	0	1(6.2)	0	0	1(6.2)	3(18.8)	1(6.2)	4(25.1)	1(6.2)	1(6.2)	5(31.3)	3(18.8)	1(6.2)	0	21(9.4)
4 *No (Rate*)*	3(19.3)	0	3(19.3)	0	1(6.4)	0	1(6.4)	1(6.4)	2(12.8)	3(19.3)	2(12.8)	0	0	1(6.4)	17(7.8)
5 *No (Rate*)*	2(12.9)	0	1(6.4)	0	1(6.4)	2(12.9)	0	1(6.4)	0	1(6.4)	0	0	0	1(6.4)	9(4.1)
6 *No (Rate*)*	0	0	0	0	0	2(12.9)	2(12.9)	2(12.9)	3(19.3)	1(6.4)	0	0	0	0	11(5.0)
7-9 *No (Rate*)*	0	0	0	1(2.1)	0	5(10.7)	1(2.1)	1(2.1)	2(4.3)	2(4.3)	1(2.1)	0	0	0	13(2.0)
10-14 *No (Rate*)*	2(2.7)	1(1.3)	0	1(1.3)	0	1(1.3)	2(2.3)	0	3(4.0)	0	0	0	0	1(1.3)	11(1.0)
15-19 *No (Rate*)*	0	1(1.1)	0	3(3.5)	2(2.3)	1(1.1)	2(2.3)	1(1.1)	1(1.1)	0	1(1.1)	0	1(1.1)	0	13(1.0)
20-29 *No (Rate*)*	17(7.6)	13(5.8)	8(3.5)	4(1.7)	1(0.44)	3(1.3)	4(1.7)	2(0.89)	2(0.89)	3(1.3)	3(1.3)	1(0.44)	3(1.3)	0	64(2.0)
30-39 *No (Rate*)*	3(1.1)	7(2.7)	1(0.3)	1(0.3)	1(0.3)	2(0.7)	4(1.5)	2(0.7)	0	1(0.3)	2(0.7)	1(0.3)	1(0.3)	0	26(0.7)
40-49 *No (Rate*)*	3(1.3)	1(0.4)	2(0.9)	1(0.4)	1(0.4)	1(0.4)	1(0.4)		3(1.3)	2(0.9)	5(2.2)	1(0.4)	1(0.4)	1(0.4)	23(0.7)
50-59 *No (Rate*)*	1(0.4)	0	2(0.8)	2(0.8)	1(0.4)	0	3(1.2)	0	0	4(1.6)	0	2(0.8)	0	0	15(0.4)
60-69 *No (Rate*)*	0	1(0.5)	0	1(0.5)	1(0.5)	0	1(0.5)	0	0	0	0	0	0	0	4(0.1)
70+ *No (Rate*)*	0	0	0	0	0	1(0.5)	0	2(1.0)	0	0	0	0	0	0	3(0.1)
(Total)	32(2.0)	26(1.63)	19(1.19)	14(0.88)	13(0.81)	22(1.33)	23(1.37)	20(1.21)	23(1.39)	25(1.51)	24(1.45)	11(0.66)	9(0.54)	4(0.24)	265(1.1)

followed by the age group of 3 (9.4/100,000) and 4 (7.8/100,000) years, and the lowest in persons aged 60 and over (0.1/100,000). Epidemiological research conducted in the European Union in 2016 indicated that the age-specific incidence rate of giardiasis was highest in the age group of 1 to 4 years (19.4/100,000), and lowest in people aged 65 and over (3.3/100,000), like the results of our study (13).

The highest percentage of giardiasis patients in Belgrade was registered in the municipalities of Savski venac 106 (44%), Voždovac 38 (14%), Novi Beograd and Zemun 21 (8%), and the lowest in the municipalities of Lazarevac and Mladenovac 1 (0.3%), Grocka and Čukarica 2 (1%). While in the municipalities of Barajevo, Sopot and Surčin, no disease was registered. Giardiasis was registered throughout the year with seasonal increases in late summer and early autumn. The highest number of patients was registered in September and October 62 (23.39%), with the appearance of a smaller peak in March 33 (12.45%), and the lowest in May and December 15 (5.66%). A similar situation was observed in 2017 in EU countries where giardiasis is registered throughout the year, with a peak in September and a smaller peak in March (10). The seasonal trend is pronounced in the United States, the number of patients increased in the summer and autumn months (from June to October), with a peak in August (14). According to the Atlanta Centers for Disease Control in the United States, giardiasis is the most common intestinal parasitic disease with over a million cases per year (15). During 2012–2017.year, public health officials from 26 countries reported 111 giardiasis epidemics (760 cases) to the National Epidemic Reporting System (NORS). Three main ways of spreading the infection have been identified: exposure to water in 29 (26%) epidemics, person-to-person contact in 28 (25%) epidemics, and contaminated food in 6 (5%) epidemics. In 48 (43%) epidemics, the route of transmission was not determined. Private homes and childcare facilities have been the most common outbreak locations for all modes of transmission (2). In comparison, in our study no epidemic of giardiasis was registered around Belgrade in the observed period from 2007 to 2020.

Conclusion

During the study period from 2007 to 2020, 265 new cases of giardiasis were registered on the territory of Belgrade. The largest number of new patients was recorded at the beginning of the study in 2007, and the smallest in the last year of the study in 2020. Among the patients, there were more males, aged 2 years old. On the territory of Belgrade, the largest number of patients was registered in the Belgrade municipalities (Savski venac, Voždovac), and in the months of early autumn (September, October), which corresponds to the seasonal distribution of giardiasis.

Epidemiological situation of giardiasis around Belgrade in the observed period shows a declining trend in the number of patients and the incidence rate, which is most likely the result of improved hygienic and sanitary living conditions. Continuous health education of the population is necessary, to be informed about the manner of transmission of giardiasis and the application of general prevention measures, as well as to raise awareness of the importance of timely reporting to the health service, at the appearance of the first symptoms of the disease due to appropriate therapy. Further research is necessary in this field to identify models with which it would be possible to monitor and understand the time characteristics of contagious diseases.

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EPIDEMIOLOŠKE KARAKTERISTIKE ĐARDIAZE NA PODRUČJU BEOGRADA U PERIODU OD 2007. DO 2020. GODINE

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Cilj ovoga rada je da se analiziraju epidemiološke karakteristike đardiaze na području Beograda u periodu od 2007. do 2020. godine. Primenjena je deskriptivna epidemiološka studija. U posmatranom periodu, na području Beograda registrovano je 265 novoobolelih od lamblijaze, a prosečna sirova incidencija iznosila je 18,92/100.000. Muškarci (56%) su češće obolevali od lamblijaze nego žene (44%). Najviša uzrasno specifična stopa incidencije zabeležena je u uzrastu od dve godine (10,3/100.000). Najveći broj obolelih registrovan je u opštini Savski venac 106 (40%), a sezonska distribucija ukazuje na to da je najveći broj obolelih registrovan u septembru i oktobru. Epidemiološka situacija đardiaze na području Beograda u posmatranom periodu pokazuje opadajuću tedenciju broja obolelih i stope incidencije, što je najverovatnije rezultat poboljšanja higijenskih i sanitarnih uslova života. *Acta Medica Medianae 2023;62(2):31-37.*

Ključne reči: *đardiaza, protozoa, dijareja, higijena*

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Original article

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ANTIMICROBIAL ACTIVITY EVALUATION OF LYOPHILIZED JUICE AND WASTE EXTRACT OF RED CURRANT VARIETY REDPOLL

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Berries include a large number of species that are known for their nutritional and health benefits. Several studies have found that red currant has different biological properties, such as antiproliferative, anticancer, antimicrobial, anti-inflammatory, antidiabetic, and antioxidant. The aim of this study was to investigate the antimicrobial activity of lyophilized fruit juice (RPJL) and waste extract (RPWL) obtained from red currant (*Ribes rubrum* L.) variety Redpoll on different Gram-positive (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Enterococcus faecalis) and Gram-negative bacteria (Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Proteus mirabilis, Enterobacter aerogenes) and one yeast (Candida albicans*). The activity of dominant active compounds (ascorbic acid, quercetin and kaempferol) contained in red currants under the same conditions were also examined to determine their responsibility in the antimicrobial effect. Tested RPJL and RPWL showed moderate antimicrobial activity. The minimum inhibitory (MIC) and microbicidal concentrations (MBC/MFC) of RPJL and RPWL were 100 mg/ml and more than 100 mg/ml. RPJL and RPWL have the same effect on Gram (+) bacteria and the same MIC and MBC value. RPWL showed stronger antimicrobial effects on Gram (-) bacteria while the juice did not inhibit the growth of the Gram (-) bacteria at all. Standard solutions of ascorbic acid and quercetin showed strong inhibitory and microbicidal activity at lower concentrations than tested samples with the MIC/MBC (MFC) =2.5−10 mg/ml. Results showed that red currants could have potential applications as natural antimicrobial agents. *Acta Medica Medianae 2023;62(2): 38-44.*

Key words: *red currants, Redpoll, antimicrobial activity, berries, preservatives.*

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Introduction

Berries include a large number of species that are known for their nutritional and health benefits. The unique color, fragrance, taste and nutritive content make these fruits valuable food sources. The most commonly investigated berries are strawberry, blueberry, raspberry, black and red currants (1).

The name “currant” was first mentioned in the ancient Greek city of Corinth and was initially used to describe small dried grapes from that region, while the origin of red currant (*Ribes rubrum* L.) comes from Western Europe (2, 3). Red currants belong to the genus Ribes which is classified in the family of Grossulariaceae according to the new taxonomy. It previously belonged to the genus of Saxifragaceae. The genus Ribes includes more than 150 species that are native to northern Europe, Asia, North and South America, and northwestern Africa (4). Currant fruits are berry-shaped, with the seeds enclosed in a fleshy pericarp. The leaves are divided into three to five lobes. Yellow-green flowers appear in April or May, they are arranged on a branch 4−8 cm high, developing three to ten red berries. The berries are born in clusters, which are attached to the branch by short stalks. The fruits nearest to the branch are the largest ones and ripen earlier, while the distal ones are smaller and ripen later. *Ribes rubrum* berries contain a flat pentagonal disk at the top, left over from the sepal. These pentagonal discs are important for the identification of archaeobotanical remains in frozen plant assemblies, as well as in differentiation from black currant (4).

Commercial cultivation of red currant began in the eighties of the XX century on the territory of western Serbia, where a very small part of the plantations are still used today (4).

The chemical composition of red currants vary depending on the variety, cultivation, location, stage of maturity, harvest and storage conditions (5, 6).

A wide range of nutrients (carbohydrates, vitamins, minerals, organic acids), as well as antioxidant components (polyphenols and vitamin C) make red currant important and useful plant species (7, 8). The primary metabolites of plants are sugars and organic acids, which determine the taste of the fruit (7). Secondary metabolism follows up the primary one and represents the transformation and catabolism of the resulting final products of primary metabolism (9). The dominant group of secondary metabolites in currants is polyphenols and their three most common classes are flavonoids, tannins and phenolic acids. Anthocyanins (delphinidin- and cyanidin-3-*O*-glucoside), flavanols (catechin, epicatechin), flavonols (quercetin, kaempferol, myricetin), condensed tannins (proantho-cyanidins), hydrolyzing tannins (ellagitannins and gallotannins) and phenolic acids (hydroxybenzoic acid and hydroxycinnamic acid derivatives) were found in red currants (6, 10-13).

According to the literature, numerous health benefits of red currants come from phenolic components. Several studies have found that they have various biological effects, such as antiproliferative, anticancer, antimicrobial, anti-inflammatory, antidiabetic, and antioxidant (14-17). Berries rich in phenolics also exhibit antimicrobial action against pathogenic bacteria (18). The antimicrobial effect of polyphenolic compounds has been demonstrated in the research of Gatto et al. (2002) and De Pascual-Teresa (2008) (19, 20). These active compounds inhibit the growth of microorganisms, but do not express microbicidal effect (21). *Salmonella, Staphylococcus, Helicobacter,* and *Bacillus* species are the most sensitive bacteria for the berry phenolics (22). In addition, the growth of *Escherichia, Clostridium* and *Campylobacter* species, but not *Lactobacillus* and *Listeria* species, is inhibited by berry phenolics (23). Liegiūtė (2006) showed that sour cherry extracts can suppress the growth of both Gram (+) and Gram (-) bacteria (24). Cranberry juice suppresses the adhesion behavior of *Escherichia coli* and also inhibits the adhesive properties of oral streptococci, thus weakening biofilm formation and mouth colonization (25, 26). The ability of *Helicobacter pylori* to colonize mucous membranes is also inhibited by cranberry extracts (27). While red currant has found its place in the food industry in the preparation of jams, juices, wine, teas and sweets, in medicine and pharmacy could be used to treat and prevent some diseases due to the high content of bioactive components (28-30).

World Health Organization revealed that 1 in 10 people become ill eating contaminated food and 420,000 die every year. Children under 5 are more affected, with 125,000 deaths every year. Food can be contaminated at any point of production and distribution, but also if improperly prepared or mishandled at home. *Salmonella, Campylobacter,* and *Escherichia coli* are among the most common foodborne pathogens that can cause most severe and fatal outcomes on millions people per year. Symptoms usually include fever, headache, nausea, vomiting, abdominal pain and diarrhea. Outbreaks of salmonellosis involve contaminated eggs, poultry and other animal products. Raw milk, raw or undercooked poultry and contaminated drinking water are the main causes of *Campylobacter* foodborne cases. Unpasteurized milk, undercooked meat and fresh fruits and vegetables can be contaminated with enterohaemorrhagic *Escherichia coli* (31).

The aim of this study was to investigate the antimicrobial activity of lyophilized fruit juice (RPJL—Redpoll juice lyophilizate) and waste (RPWL—Redpoll waste lyophilizate) obtained from red currant (*Ribes rubrum* L.) variety Redpoll on different Gram-positive and Gram-negative bacteria and yeast (*Candida albicans*). The second aim was to examine the activity of standard compounds that are found in red currants under the same conditions and to determine their responsibility in the antimicrobial effect. The results could be useful for the food industry where red currants extracts could be used as potential natural preservers.

Material and methods

Plant material and sample preparation

Red currant variety Redpoll was grown and collected from Radmilovac, experimental field of the Faculty of Agriculture, University of Belgrade. Fully ripe berries were harvested from the end of June to the beginning of July of 2020. After picking, the fruits were thawed and pressed in a special press for squeezing fruit, obtaining the juice for further analysis. The residue after straining (waste) was dried on filter paper for one day, after which it was dried in a laboratory dryer at 40°C for 48 hours. The residue left after drying was grinded in a mill. The maceration method was used for extraction of the plant material. In the maceration process, 60% ethanol was used, with a material to extragens ratio of 1:20. The sample was extracted on a laboratory shaker for 60 min at room temperature. After extraction, the ethanol was evaporated using a rotary evaporator.

Antimicrobial activity evaluation

The antimicrobial activity of RPJL and RPWL was assessed using laboratory control strains obtained from the American Type Culture

Collection (ATCC). Gram (+) bacteria used in this assay were *Bacillus cereus* ATCC 10876, *Listeria monocytogenes* ATCC 15313, *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 19433. Gram (−) bacteria were represented by *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteritidis* ATCC 13076, *Proteus mirabilis* ATCC 12453, and *Enterobacter aerogenes* ATCC 13048. *Candida albicans* ATCC 15313 was used for assessing antifungal activity.

The bacterial and fungal inocula were made from overnight broth cultures. The suspensions with the microorganisms were adjusted to 0.5 McFarland standard turbidity (which corresponds to 107−108 colony forming units (CFU/ml) for bacteria depending on genera and 0.4×105 to 5×105 spore/ml for fungal strains (consensus standard by the National Committee for Clinical Laboratory Standards (NCCLS)) (32). The determination of antimicrobial and antifungal activity (the minimum inhibitory concentration [MIC] and minimum bactericidal/fungicidal concentration [MBC/MFC]) was carried out by microwell dilution method, according to the NCCLS (2003). Tested samples were dissolved in 10% DMSO. The final concentrations of ascorbic acid, quercetin and kaempferol were 20, 10 and 5 mg/ml in 10% DMSO. Serial doubling dilutions of the tested samples and standards were prepared in the 96-well microtiter plates in inoculated nutrient broth. The final volume was 100 µl and the final bacterial concentration was 2×106 CFU/ml in each well and 2×105 of spores for fungal strains. The plates were incubated for 24h at 37oC for bacteria and 48 h at 25ºC for fungi. Microbial growth was determined by adding 20 µl of 0.5% triphenyltetrazolium chloride aqueous solution (33). The minimal concentration where there was no visible growth was defined as the minimal inhibitory concentration (MIC). For MBC/MFC determination, the broth was taken from each well and inoculated into Mueller Hinton agar at 37ºC for 24h for bacteria, or in malt extract agar at 25ºC for 48h for fungal strains. The minimal bactericidal/fungicidal concentration (MBC/MFC) was defined as the lowest concentration of the sample that had killed 99.9% of microorganism cells (32). Doxycycline and nystatin were used as the controls.

Results and Discussion

The antimicrobial activity of tested samples and standards against selected microorganisms is shown in Table 1. Tested RPJL and RPWL showed moderate antimicrobial activity. In this study, red currant RPJL did not inhibit Gram (-) bacteria (*E. coli, P. aeruginosa S. enteritidis, P. mirabilis*, and *E. aerogenes*), while was effective on all Gram (+) bacteria (*B. cereus, S. aureus, E. faecalis*), except *L. monocytogenes*. As other researchers have determined when it comes to the antibacterial effects of herbal extracts, the effect on Gram (-) bacteria is significantly lower compared to Gram (+) ones (34). Red currant RPWL inhibited activity of both Gram (+) and Gram (-) bacteria, except *E. coli*. The minimum inhibitory and microbicidal concentrations, MIC/MBC (MFC) of RPJL and RPWL were 100 mg/ml and more than 100 mg/ml. RPWL shown stronger antimicrobial effects compared to RPJL, especially on Gram (-) bacteria.

The significance of the bacteriostatic activity of RPJL and RPWL against *B. cereus* is that they can stop this causative agent of alimentary infections accompanied by nausea and vomiting (35). Inhibitory activity of RPWL against *L. monocytogenes* may be helpful in preventing listeriosis, which is also associated with food contamination (36). RPJL didn't have any effect on the *L. monocytogenes*. In this study, neither RPWL nor RPJL showed activity against *E. coli*, which is responsible for urogenital tract infections, meningitis, pneumonia and sepsis (37).

In this research, standard solutions of ascorbic acid and quercetin showed strong inhibitory and microbicidal activity at lower concentrations than tested samples. The MIC/MBC (MFC) of standards were 2.5−>10 mg/ml. Kaempferol showed no activity, except on *S. aureus*, where MIC/MBC were 2.5/>2.5. There are literature data showing results of kaempferol esters antimicrobial activity on four bacteria (*E. faecalis, S. aureus, E. coli, P. aeruginosa*). MIC values varied from 23−250 µg/ml (38). The difference between the results can be attributed to a different type of sample as well as the different methoxylation patterns. Kaempferol with free -OH groups (without any methoxyl substitution) was the most active.

Values of MIC/MBC for ascorbic acid in our study were 2.5−5/>10.0 mg/ml. One study reported that application of low concentration of vitamin C (0.15 mg/mL) inhibited the growth of *S. aureus* and *E. faecalis* (39). *C. albicans* was more sensitive to the ascorbic acid (10/>10 mg/ml) then to RPJL and RPWL. Quercetin and kaempferol did not inhibit the growth of *C. albicans*.

The literature data on the antibacterial activity of lyophilized juices and waste are scarce, while the results on the effect of extracts of red currants are limited. Data on antimicrobial and antifungal activities of red currants are different. There is a study that examined the inhibition of the growth of Gram (+) and Gram (-) bacteria by the action of juice of different fruits. They showed that red currant juice had antibacterial effect on most of the oral bacterial species tested (*S. gordonii, S. sobrinus, F. nucleatum, A. actinomycetemcomitans, Pseudomonas gingivalis, E. faecalis*), with none, or only very limited cytotoxic effects on human gingival fibroblasts (40). Also, another study shown that extracts with low or medium total phenolics and low anthocyanins were the strongest suppressors of *S. aureus* and *Lactococcus lactis* subsp. *Lactis* (41).

Table 1. Antimicrobial activity of lyophilisates of Redpoll juice (RPJL) and waste (RPWL) and standard substances (ascorbic acid, quercetin and kaempferol) against pathogenic microorganisms (MIC/MBC in mg/mL)

EXTRACTS		**RPJL**	**RPWL**	**Ascorbic acid**	**Quercetin**	**Kaempferol**	**Doxycycline**	**Nystatin**
Bacterial strains	**Source**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (μg /ml)**	
Gram (+)	**ATCC**							
Staphylococcus aureus	6538	100.0/>100.0	100.0/>100.0	2.5/>10.0	2.5/>5.0	2.5/>2.5	7.81/15.61	
Enterococcus faecalis	19433	100.0/>100.0	100.0/>100.0	2.5/>10.0	2.5/>5.0	nd	0.90/1.90	
Bacillus cereus	10876	100.0/>100.0	100.0/>100.0	2.5/>10.0	5.0/>5.0	nd	0.90/15.61	
Listeria monocytogenes	15313	nd	100.0/>100.0	2.5/>10.0	nd	nd	7.81/15.61	
Gram (-)	**ATCC**							
Pseudomonas aeruginosa	27853	nd	100.0/>100.0	2.5/>10.0	5.0/>5.0	nd	15.61/15.61	
Escherichia coli	25922	nd	nd	5.0/>10.0	5.0/>5.0	nd	15.61/15.61	
Enterobacter aerogenes	13048	nd	100.0/>100.0	5.0/>10.0	5.0/>5.0	nd	7.81/15.61	
Salmonella enteritidis	13076	nd	100.0/>100.0	2.5/>10.0	5.0/>5.0	nd	0.90/1.90	
Proteus mirabilis	12453	nd	100.0/>100.0	5.0/>10.0	5.0/>5.0	nd	7.81/15.61	
Fungal strain	**Source**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (mg/ml)**	**MIC/MBC (μg/ml)**
Yeast	**ATCC**							
Candida abicans	15313	100.0/>100.0	100.0/>100.0	10.0/>10.0	nd	nd	nd	16.0/16.0

Conclusion

We can conclude that RPJL and RPWL have the same effect on Gram (+) bacteria and the same MIC and MBC values. The difference between these two forms is that the juice does not inhibit the activity of Gram (-) bacteria. Standard solutions of ascorbic acid and quercetin showed strong inhibitory and microbicidal activity.

Based on these results, red currants could have important applications as natural antimicrobial agents. Lyophilized juice and waste of red currant (*Ribes rubrum* L.) variety Redpoll might be used as a potential preservative in the food industry.

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Procena antimikrobne aktivnosti liofilizata soka i ekstrakta ostatka ploda crvene ribizle sorte *Redpoll*

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Bobičasto voće sadrži veliki broj vrsta poznatih po svojim nutritnivnim i zdravstvenim benefitima. Crvena ribizla pokazuje različite biološke aktivnosti: antiproliferativnu, antikancerogenu, antimikrobnu, antiinflamatornu, antiokisativnu i antidijabetičku akitvnost. Cilj ovog rada bio je da se ispita antimikrobna aktivnost liofiliziranog soka crvene ribizle vrste *Redpoll* (RPJL) i liofilizovanog ekstrakta ostatka (RPWL) na različite Gram (+) bakterije (*Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Enterococcus feacalis*), Gram (-) bakterije *(Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis, Proteus mirabilis, Enterobacter aerogenes*) i jednu gljivicu (*Candida albicans*). Drugi cilj bio je ispitati aktivnost dominantnih bioaktivnih jedinjenja (askorbinska kiselina, kvercetin i kempferol), koja se nalaze u crvenoj ribizli, pod istim uslovima, kako bi se procenio njihov efekat u antimikrobnoj aktivnosti. Testirani RPJL i RPWL pokazali su umerenu antimikrobnu aktivnost. Minimalne inhibitorne (MIC) i mikrobicidne koncentracije (MBC/MFC) RPJL i RPWL bile su 100 mg/ml i više od 100 mg/ml. RPJL I RPWL pokazali su isti efekat na Gram (+) bakterije i istu MIC i MBC vrednost. RPWL je pokazao jači antimikrobni efekat na Gram (-) bakterije, dok sok uopšte ne inhibira rast Gram (-) bakterija. Standarni rastvori askorbinske kiseline i kvercetina pokazali su jaku inhibitornu i mikrobicidnu aktivnost pri nižim koncentracijama od ispitivanih uzoraka. MIC/MBC (MFC) standarda bili su od 2,5 mg/ml do 10 mg/ml. Rezultati su pokazali da bi ekstrakti i sokovi crvene ribizle mogli biti upotrebljeni kao potencijalni prirodni konzervansi. *Acta Medica Medianae 2023;62(2):38-44.*

Ključne reči: *crvena ribizla, Redpoll, antimikrobna aktivnost, bobičasto voće, konzervansi*

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Original article

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MATRIX METALLOPROTEINASES: IMPORTANT PARTICIPANTS IN EVERY STEP OF TUMOR DEVELOPMENT AND PROMISING TARGETS IN MODERN ANTITUMOR THERAPIES

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Matrix metalloproteinases are proteolytic enzymes that are able to cleave almost all components of extracellular matrix as well as many other soluble and membrane attached molecules of very diverse nature. Their proteolytic activity is crucial for embryogenesis, tissue development, remodeling and organisation. As important as in physiological processes, they play crucial role in tumor development, progression, tissue invasion and metastasis.

In this review, we discuss complex involvement of these zinc-dependent endopeptidases in every step of tumor development and progression. We highlight the importance of collaboration between tumor cells and tumor microenvironment at different levels of tumor development and spreading. We also emphasize the importance of inhibition of certain matrix metaloproteinases (depending on tumor type and stage) in order to support cytostatic therapy. *Acta Medica Medianae 2023;62(2): 45-51.*

Key words: *matrix metalloproteinases, tumor microenvironment, stem cells, angiogenesis, invasion*

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Introduction

Matrix metalloproteinases (MMPs) are family of zinc-dependent endopeptidases (1) that are capable of cleaving almost every component of the extracellular matrix (ECM). According to their substrate specificity and their primary structure, they can be divided into subgroups: collagenases, gelatinases, stromelysins, membrane-associated MMPs (Table 1).

All MMPs possess basic domain structure: 1) signal peptide for extracellular localization; 2) prodomain that inhibits the zymogen form and 3) conserved catalytic domain. Some MMPs possess additional domains that enable localization, interaction with protein complexes or selectivity for specific proteins (2). They are synthesized as inactive zymogens and can be activated either by other MMPs or by serine proteases.

MMPs are crucial for organ development during embryogenesis (3, 4), healing processes, tissue regeneration, angiogenesis and many other processes that include rearrangement of extracellular matrix (ECM) and modulation of signaling pathways (5). Due to their ability to interact with multiple substrates in a proteolytic manner, MMPs can control cell adhesion, migration and stem cell differentiation (6-8). As important as in physiological processes, they also play crucial role in tumor development, invasiveness and metastases (9). Earlier researches were based on the ability of MMPs to degrade components of ECM (10) and enable invasion and metastases of cancer cells (11). Furthermore, it was established that the role of MMPs in tumor development is much wider and much more complicated. They are also involved in cell proliferation, apoptosis, angiogenesis and epithelial to mesenchymal transition (EMT) (12-14). Due to their ability to cleave different molecules, such as growth factors, cell surface receptors, cell adhesion molecules, cytokines or chemokines, MMPs can also modulate signaling pathways that regulate differentiation and modulate stem cell niches (15).

MMPs can be derived from tumor cells and also from tumor microenvironment (host derived),

Table 1. Classification of matrix metalloproteinases

MMP family	Name	Number
Collagenases	Interstitial collagenase	MMP-1
	Neutrophil collagenase	MMP-8
	Collagenase 3	MMP-13
Gelatinases	Gelatinase A	MMP-2
	Gelatinase B	MMP-9
Stromelysins	Stromelysin-1	MMP-3
	Stromelysin-2	MMP-10
	Stromelysin-3	MMP-11
	Stromelysin-4	MMP-19
Matrilysins	Matrilysin-1	MMP-7
Elastase	Matrilysin-2	MMP-26
	Metalloelastase	MMP-12
Membrane type	MT1-MMP	MMP-14
	MT2-MMP	MMP-15
	MT3-MMP	MMP-16
	MT4-MMP	MMP-17
	MT5-MMP	MMP-24
Unclassified	MT6-MMP	MMP-25
	Enamelysin	MMP-20
		MMP-18
		MMP-23
	Epilysin	MMP-28

such as cancer activated fibroblasts (CAFs) or inflammatory cells. Actually, the interaction between tumor cells and tumor microenvironment is crucial for tumor promotion, invasion and progression (16, 17).

The role of MMPs in tumor initiation process

High degree of genetic heterogeneity in tumors suggest that genomic instability is crucial for tumor initiation and development. By cleaving the components of ECM, MMPs can start tumor initiation and progression because the instability of ECM can indirectly activate cellular processes that cause genomic instability and DNA damage (18). MMPs target molecules involved in cell-ECM adhesion or cell-cell adhesion, such as E-cadherin. Some researches showed that loss of cell adhesion reduces expression of p53 in diverse cell types. In keratinocytes, the loss of cell adhesion reduces p53 expression by 80% (19). Adhesion dependent loss of genomic surveillance increases DNA damage and induces genomic instability. Rac1b was identified in breast and colorectal tumors and is involved in accumulation of cyclin D1, cell cycle progression, apoptosis resistance and cellular transformation. Production of Rac1b is also involved in elevating levels of reactive oxygen species (ROS) that can directly damage DNA or DNA repairing mechanisms (20, 21). It was also shown that MMP3 stimulates production of Rac1b in breast cancer. Sustained expression of MMP3 by breast stromal cells leads to hyperplasia, dysplasia and other changes in stromal components (22).

Effects of MMPs on proliferation and apoptosis

MMPs can activate TGF-β in a proteolytic manner (by cleaving their precursor) or in a non proteolytic manner (by modifying the ECM or though HPX domain). In normal tissue, TGF-β has proapoptotic and cytostatic function, but in cancer tissue, its action is reversed. MMPs can also proteolytically activate growth factors (EGF, HGF, IGF...) and promote tumor expansion (23, 24). On the contrary, they can inactivate Fas receptor and disable proapoptotic pathway, which leads to inhibition of apoptosis and consequent resistance to chemotherapy. MMP-7 plays an important role in activation of EGFR and also cleaves cell surface proteins such as Fas ligand and E-cadherin, which promotes cellular proliferation and disables apoptosis (25).

MMP take role in epithelial-to-mesenchymal transition

Epithelial-to-mesenchymal transition (EMT) is a key step in tumor invasion and metastasis. It is a process during which epithelial cells loose cell to cell adhesion and polarity and enhance their motility (26). This process is characterised by

upregulation of mesenchymal markers and N-cadherin and downregulation of E-cadherin. MMP-induced EMT has been studied in many different cancer tissues. MMP-3 directly activates EMT process in breast cancer. MT1-MMP induces EMT in esophageal and oral squamous cell carcinoma, breast carcinoma and prostatic carcinoma (27). Upregulation of MMP-7 leads to acinar-to-ductal metaplasia, a precursor of pancreatic ductal adenocarcinoma through activation of Notch signaling pathway (28). MMP-1 can activate proteinase activated receptor (PAR) by cleaving its extracellular domain. This leads to cancer cell migration and invasive behaviour in breast cancer (29).

MMP induced EMT can increase tumorigenicity through induction and regulation of cancer stem cell characteristics.

Cancer stem cells represent small population of tumor cells responsible for tumor growth, heterogeneity, metastatic potential and resistance to chemotherapy. In previous studies has been established that tumor cells with stem cell like characteristics showed asymmetric division, very slow replication and resistance to chemotherapy and apoptosis (30). Cancer stem cells as well as stem cells in normal tissue are placed in stem cell niche which consists of extracellular matrix, adjacent stromal cells and extracellular soluble factors: cytokines, chemokines, growth factors... The niche provides balance between quiescence and self-renewal of stem cell population and prevents uncontrolled proliferation.

Thanks to their ability to cleave and degrade different components of ECM, MMPs can disturb the balance within the stem cell niche (31). The best-studied influence of MMPs on stem cell niche is the one in the bone marrow: MMP-9, MMP-14, MT1-MMP (32, 33). In human epidermal cells, inhibition of MMP-2 and MMP-14 provides longer cell survival (34, 35). MMP-10 leads to the expansion of bronchoalveolar stem cells in the context of K-ras derived lung carcinoma (36). It was also shown that MMP-10 regulates stemness of ovarian cancer stem-like cells and its overexpression leads to maintenance of cancer stem cells (CSCs) and resistance to platinum reagent (37).

MMPs influence tumor angiogenesis

During angiogenesis, quiescent endothelial cells (ECs) become migratory and invade the surrounding tissue. This process requires enzymes that are able to cleave components of basement membranes (BM) and ECM. Many performed studies proved that MMP-9 is a critical component of angiogenic switch. MMP-9 is able to activate vascular endothelial growth factor (VEGF) and start the cascade of events that lead to endothelial cells proliferation, migration, survival and new vessels formation. Other studies implicated that MMP-9 can also act as an inhibitor of angiogenesis through activation of angiogenesis inhibitors: angiostatin, tumastatin, endostatin (38). Some authors tried to explain these contradictory findings by the origin of MMP-9 and by the diversity of models. In mouse models of chondrosarcoma cells it was shown that downregulation of MMP-2 resulted in suppression of tumor growth through reduced angiogenesis. There are also studies that highlight the role of membrane type metalloproteinase MT1-MMP in tumor angiogenesis (39, 40).

MMPs enable tissue invasion and metastasing

Degradation of stromal connective tissue and basement membrane are two crucial processes in tumor invasion and metastases. Interstitial collagens are extremely resistant to proteolytic attacks and can be degraded only by MMPs (41). MT1-MMP (MMP14) is well established and most important in pericellular proteolysis. Overexpression of MT1-MMP in squamous cell carcinoma (SCC) of oral cavity and oesophageal SCC leads to higher invasiveness and poor prognosis (42). MMP-9 and MMP-11 were shown to be significantly prognostic for shorter relapse-free survival in breast cancer. Serum levels of MMP-2, MMP-9 and MT1-MMP are significantly higher in patients with bone metastases in prostate cancer (43). MMP-13 can activate proteolytic cascade that enables activation of MMP-9 and cleavage of galectin, a suppressor of osteoclastogenesis. This event promotes osteoclastogenesis, and creates favorable microenvironment for bone metastasis in breast cancer (44).

The most studied MMPs whose overexpression correlates with poor prognosis in different types of carcinoma are shown in Table 2.

Possibilities for therapeutic inhibition of MMPs

There are several MMP inhibitors that take part in different processes: physiological or pathological. There is a circulating general protease inhibitor alpha-2-macroglobulin and four tissue inhibitors of metalloproteinases (TIMPs). In physiological circumstances, these inhibitors provide balance between degradation and production in ECM. They are non-selective and all MMPs can be inhibited by a number of different TIMP proteins (45). So far, attempts for MMP inhibition by TIMPs in order to prevent tumor progression revealed mostly unsuccessful. One of possible explanations is that not all MMPs are related to poor prognosis. For example, MMP-8 was shown to be cancer-protective MMP and its downregulation is associated with poor outcome in breast cancer (46) and melanoma (47).

Table 2. The most studied MMPs related to poor prognosis in different types of carcinoma

Tumor type	MMPs related to poor prognosis	Reference
Lung carcinoma	MMP-2, MMP-3, MMP-9, MMP-10	17, 36
Breast cancer	MMP-3, MMP-7, MMP-9 MMP-11, MT1-MMP, MT2-MMP	18, 19, 21, 22, 25
Epithelial ovarian cancer	MMP-10	37, 48
Oral squamous cell carcinoma	MT1-MMP, MMP-7	42
Oesophageal squamous cell carcinoma	MMP-1, MMP-2, MMP-3, MMP-7, MMP-9, MMP-10, MT1-MMP	27
Colorectal carcinoma	MT1-MMP, MMP-1, MMP-2, MMP-3, MMP-7, MMP-11	6, 9
Prostate cancer	MMP-2, MMP-9, MT1-MMP	11, 43, 45
Pancreatic carcinoma	MMP-7	28

Marimastat was the first orally bioavailable MMP inhibitor to enter clinical testing. Trials were conducted in patients with colorectal, ovarian, pancreatic and hormone- refractory prostate cancer. Although phase I and II were promising, there are no precise data about phase III trials to date.

Preclinical studies with batimastat—competitive, reversible, broad-spectrum MMPI were performed in mice and suggested favorable therapeutic effects in ovarian, breast, colon cancer treatment as well as in treatment of melanoma, in combination with standard therapies. The most dramatic effect has been accomplished in ovarian carcinoma concurrent treatment with cisplatin and batimastat (48).

Studies with resveratrol (RSV) in cell lines and mouse models were also successful. RSV is natural product whose spectrum of influence is much wider than only MMP inhibition. Studying its effect in prostatic cancer development it was shown that RSV may inhibit cancer initiation, proliferation and metastases in many levels by targeting tumor microenvironment (TME) (49, 50).

Conclusion and further perspectives

Tumor development and progression is multi-leveled process that involves the interaction of tumor cells and host microenvironment. Interaction between tumor cells and host components such as immune cells, fibroblasts, endothelial cells, components of ECM is necessary for tumor growth at primary site, invasion and progression as well as metastasing to distant organs.

Matrix metalloproteinases, as powerful proteolytic enzymes, derived from tumor cells and also from host cells, contribute to multiple stages of tumor progression. Through their ability to degrade basement membrane, components of ECM and nonmatrix substrates enable tumor spreading and help tumor angiogenesis. Their role in maintenance of cancer stem cells is also important and contributes to resistance to chemotherapy and apoptosis.

As important as destruction of tumor cells by chemo and radiotherapy, it is also necessary to block the interaction between tumor cells and tumor microenvironment, because tumor microenvironment enables maintenance and survival of tumor cells. Compromising the expression of wide range of MMPs is a logical and promising step in anti-cancer therapy to support standard therapies.

Trials with TIMPs were not so successful and differed in cell culture and animal models. The reason might be in non-selectivity of these inhibitors. Nowadays, the understanding of how each MMP acts in different cancers and different stages of cancer development and progression is much more sophisticated. In combination with newly developed methods for discovering highly selective inhibitors of MMPs it can be a promising step forward in attempts to disable communication between cancer cells and their microenvironment. Targeting multiple proteases may be an effective strategy for stopping tumor growth and progression. All of this could lead to much more successful, combined anticancer therapies.

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MATRIKS METALOPROTEINAZE – VAŽNI UČESNICI U SVAKOM KORAKU RAZVOJA TUMORA I OBEĆAVAJUĆE METE U SAVREMENOJ ANTITUMORSKOJ TERAPIJI

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Matriks metaloproteinaze su proteolitički enzimi koji mogu da razgrađuju skoro sve komponente ekstracelularnog matriksa, kao i mnoge druge solubilne i membranske molekule različite prirode. Njihova proteolitička aktivnost veoma je značajna za embriogenezu, razvoj, remodelovanje i organizaciju tkiva. Osim što su veoma značajne za odvijanje fizioloških procesa, imaju i veoma važnu ulogu u razvoju i progresiji tumorskog procesa, tkivnoj invaziji i metastaziranju tumora.

U ovom preglednom članku razmatramo kompleksno učešće ovih endopeptidaza zavisnih od cinka u svakom koraku razvoja i progresije tumorskog procesa. Posebno ističemo značaj saradnje između tumorskih ćelija i njihove mikrookoline na različitim nivoima razvoja i širenja tumorskog procesa. Takođe, naglašavamo značaj inhibicije pojedinih matriks metaloproteinaza (u zavisnosti od vrste i stadijuma tumora) u cilju podrške citostatskoj terapiji. *Acta Medica Medianae 2023;62(2):45-51.*

Ključne reči: *matriks metaloproteinaze, tumorska mikrookolina, matične ćelije, angiogeneza, invazija*

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THE ROLE OF CYTOKINES IN SCHIZOPHRENIA

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Schizophrenia, a multisystem condition with an unclear cause, is linked to immunological dysfunctions, including altered cytokine levels. The possible role that inflammation could play in psychiatric diseases has been the subject of a growing corpus of research in the last 20 years. Individuals with schizophrenia have abnormal cytokine synthesis, abnormal cytokine concentrations, and altered cytokine receptors in their blood and cerebrospinal fluid, suggesting a relationship between inflammation and schizophrenia. Contradictory results have been observed in psychosis, leaving the pathophysiological function of inflammation in psychosis unclear. The population with chronic schizophrenia has been extensively investigated. Still, the group with first-episode psychosis (FEP) provides a unique chance to assess the biological, clinical, and functional consequences of psychotic illnesses. Results regarding cytokine concentration are inconsistent, which is a consequence of different research methodologies. However, it was found that there was a relationship between inflammation markers and disease symptoms. The development of biomarkers as quickly as possible following the onset of a disease might open the way for early disease prevention, which improves the prognosis. Intervention at an early stage stops the progression of the disease and enhances treatment outcomes. The drug-free FEP population is receiving a growing amount of attention from researchers who are conducting studies on a large scale. *Acta Medica Medianae 2023;62(2): 52-60.*

Key words: *psychosis, inflammation, schizophrenia, biomarkers*

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Introduction

Approximately 1 in 300 people worldwide have schizophrenia, a persistent and frequently debilitating mental condition that typically develops in late adolescence or early adulthood. It is characterized by positive (delusions, hallucinations and disorganization), negative (such as apathy, avolition or alogia), affective and cognitive symptoms (1, 2) and it is linked with higher mortality rates, severe cognitive impairment, and poor quality of life (3). Over the last two decades, there has been a greater emphasis on early intervention treatments for first-episode psychosis (FEP) (4). A first episode of psychosis (FEP), an acute time during which a person loses some sense of reality, is typically brought on by risk factors for schizophrenia. This may include sporadic hallucinations, delusions, and behavioral, cognitive, and functional disturbances. After receiving treatment, most people experience an initial remission of symptoms. However, symptoms remain and recur for one in three people, which raises the possibility of developing a more persistent condition (5). The clinical progression following a FEP was protracted and unpredictable, resulting in major quality of life losses for patients and their families as well as huge societal expenses. This clinical development accounts for 10% of the global burden of mental diseases in Europe (6).

Inflammation is regarded as a contributory or mediating component in the start of schizophrenia, even though genetic susceptibility and environmental stresses in the earliest years of life are crucial for the course of schizophrenia (7, 8). Neuroinflammation can damage white matter and dysconnectivity, leading to schizophrenia symptoms (9). It is well-established that a

dysregulated immunological response is associated with psychosis. Systemic inflammation can exert a major effect on the brain, as demonstrated by a growing body of studies linking inflammation and immunology to the development of schizophrenia symptoms, resulting in emotional, cognitive, and behavioral abnormalities. A potential link between inflammation and both FEP and schizophrenia has been proposed for decades (10, 11).

In this regard, the peripheral immune system-to-brain communication pathways have been widely investigated in several neuroinflammatory diseases in which inflammatory cytokines are believed to play a significant role (12, 13). It has been hypothesized that the immune responses of the periphery are reflective of the neuroimmune status of the brain. As a result, the concentrations of systemic cytokines are becoming increasingly significant for showing how inflammation may bestow particular symptom patterns in FEP (14).

Cytokines are small proteins that profoundly impact nearly every aspect of biology, from embryonic development and disease etiology to particular and immunological responses that are not specific, cognitive function, and the course of degenerative aging. They play an important role in coordinating inflammation and its response. From the perspective of the pathways they trigger, cytokines are mainly classified as pro-inflammatory and anti-inflammatory (15, 16). Given the powerful impact of cytokines, it is becoming evident that abnormalities in cytokine production, release, and signaling can play a role in the development of many different human illnesses.

The majority of research on schizophrenia and associated disorders is conducted on small patient populations. They refer to patients with a drug-naive first psychotic episode (FEDN), whose condition was monitored before administering antipsychotics to therapy. The following research cohort consists of patients with a first psychotic episode (FEAP) taking antipsychotics. The third category comprises those with subsequent psychotic episodes (relapse), followed by schizophrenia patients in stable remission. Less frequently studied patient groups include those with early-onset psychosis and those at clinically high-risk or ultra-high-risk for psychotic illness.

Interleukin 1 (IL – 1β)

In response to lipopolysaccharide (LPS), various cytokines, and complement fragments, the cytokine IL-1ß, a member of the IL-1 family, is largely secreted by monocytes, macrophages, microglia, and lymphocytes. IL-1ß also plays a role in the complement system. It is regarded as one of the most significant pro-inflammatory cytokines (17). Multiple studies indicate that IL-1ß plays a significant role in the etiology and pathophysiology of schizophrenia. Patients with schizophrenia have an elevated release of IL-1β by peripheral monocytes prior to therapy, which is thereafter normalized by antipsychotics (18). Most patients with FEDN (19), adult and pediatric FEAP (20), and patients with chronic illness who were stable, undergoing an acute relapse, or recovering from one (11, 21, 22) had elevated levels of IL-1ß. On the other hand, many studies indicated an increase in IL-1ß levels, the 2008 meta-analysis found no significant changes in IL-1ß levels in vivo and in vitro serum concentrations (23). Serum concentrations of IL-1ß are significantly elevated in patients with acute phases of schizophrenia, independent of age or illness duration (24). Schizophrenia has been linked to a malfunction of dopaminergic and glutamatergic circuits in the brain, and it is known that IL-1 can increase the dopaminergic differentiation of rat mesencephalic progenitor cells (25). It is unclear whether IL-1ß activation causes schizophrenia or results from dopaminergic or glutamatergic dysfunction. Those who have been ill for more than six years and who have a chronic illness have not been found to have a significantly elevated level (26). Of note, a recent study discovered lower IL-1ß levels in FEDN patients with an illness duration of fewer than two years (22). IL-1ß peripheral levels are related to the severity of positive and negative symptoms and the overall psychopathological presentation (16).

Interleukin 2 (IL-2)

IL-2 is produced by T-helper type 1 and cytotoxic T (Tc) cells in response to CD28 activation. Its anti-inflammatory impact is secondary to its initial pro-inflammatory effect. In addition, IL-2 stimulates the production of IL-6, interferon (IFN), and additional inflammatory mediators (17). Several studies reported that higher levels of IL-2 were associated with fewer negative symptoms and enhanced cognitive performance. If true, IL-2 could play a crucial role in the pathophysiology of schizophrenia (10, 27). IL-2R, a signaling receptor expressed on T cells, has been found to be overexpressed in schizophrenia patients (28). It is described that soluble IL-2R levels are elevated in both treatment-naive and treatment-free patients with schizophrenia and also in patients with acute and chronic disease (29, 30). A positive connection between symptom severity and IL-2R levels supports its function in schizophrenia. IL-2R levels may represent a biomarker for individuals with treatment-resistant psychosis, and a positive correlation with symptom severity and IL-2R levels indicates its role in schizophrenia (31). Several studies have found inconsistent results with those mentioned above. According to them, IL-2 levels do not change in schizophrenia individuals. Patients with FEDN, FEAP, and chronic patients experiencing an acute relapse, recovering from it,

or being stable participated in these studies (11, 19, 21).

Interleukin 3 (IL-3)

Chronic schizophrenia individuals have been shown to have aberrant IL-3 levels (32, 33). Fu et al. observed that the IL-3 levels in FEDN patients were significantly lower than those of healthy control subjects and chronically treated schizophrenic patients (34). Similar to what was found in earlier studies, the IL-3 levels and IL-3-like activity (IL-3-LA) were significantly higher in chronically medicated patients with schizophrenia than in control subjects (32, 33). These higher levels of IL-3 may be related to illness progression and antipsychotic treatment. There was a substantial correlation between the Positive and Negative Syndrome Scale (PANSS) general psychopathology subscales and IL-3 in medicated patients with schizophrenia. In contrast, no connection between IL-3 and any clinical psychopathology was identified in FEDN individuals. Decreased IL-3 levels in FEDN patients may be associated with neuronal death and aberrant early brain development, which may contribute to the onset of schizophrenia.

Interleukin 4 (IL-4)

Even though Goldsmith et al. found decreased levels of IL-4 in FEDN patients (11), other meta-analyses, including two based on much larger samples, demonstrated that its levels were unaffected compared to healthy controls (19, 35, 36). In addition, lower IL-4 peripheral levels were found in chronic patients experiencing an acute relapse. This meta-analysis of IL-4 blood levels in this population of patients (11) was the only one conducted. No changes in IL-4 levels were detected in the population with stable schizophrenia (11) or individuals with clinical high risk (37). There was a positive correlation between IL-4 peripheral concentration, the intensity of negative symptoms, and the occurrence of depressive symptoms (16). In addition, pediatric FEAP patients (38) and adult chronic patients taking clozapine were observed to have increased levels (39).

Interleukin 5 (IL-5)

Long-term adult patients who had failed therapy multiple times and FEP children who predominantly took antipsychotics had elevated IL-5 levels (38, 40).

Interleukin 6 (IL-6)

Another important cytokine that promotes inflammation is IL-6, produced by macrophages, monocytes, and microglia. Tumor necrosis factor (TNF), interferons, lipopolysaccharide, and viral infections stimulate the secretion of IL-6 (17). C-reactive protein (CRP), which may influence the permeability of the BBB and the proliferation of microglia, is one of the acute-phase proteins whose synthesis is enhanced by IL-6 (17, 37). Reduced glutamate reuptake and disruption of neurogenesis may result from changes in IL-6 levels (41). IL-6 can be regarded as a schizophrenia "state marker." In this context, the blood levels of IL-6 and sIL-6R are higher in schizophrenia patients (19, 42). A substantial number of studies, including meta-analyses, reported elevated levels of IL-6 in several patient categories, including FEDN and FEAP patients (11, 19–21, 38). According to studies, IL-6 levels are elevated in FEP and acute relapse patients, however, normalize following antipsychotic therapy. There is a correlation between treatment-resistant schizophrenia and elevated levels of IL-6 (43), as well as between IL-6 concentration and illness duration (44). The level of IL-6 in the blood is associated with both negative and positive symptoms, as well as general psychopathological manifestations and cognitive deficits (16, 45). Interestingly, Miller at al. (21) also revealed a negative connection between IL-6 CSF levels and the severity of schizophrenia symptoms. There is a correlation between treatment-resistant schizophrenia and elevated levels of IL-6 (43), as well as between IL-6 concentration and illness duration (46). In contrast, some investigations showed no significant differences in IL-6 levels among schizophrenia individuals (47, 48).

Interleukin 8 (IL-8)

Serum IL-8 levels are higher in schizophrenia patients and associations with serum IL-2 or IL-8 concentrations at baseline and therapy efficacy have been found (49). Compared to the control group, patients with the diagnosis of paranoid schizophrenia exhibited statistically significant elevations in serum IL-8 levels (50, 51). In FEDN (21, 35) and relapsed chronic patients with schizophrenia (11, 21, 52) but not in FEAP (21, 35), peripheral levels persistently increased (53). In contrast, neither clinically high-risk nor ultra high-risk populations show any changes (38, 39). Western blotting has demonstrated that the expression of IL-8 in the brain tissue of schizophrenia patients is much higher than that of healthy controls. This high IL-8, IL-6, and TNF levels imply that schizophrenia may be an autoimmune neuropsychiatric spectrum disorder which may occur during an autoimmune CNS disease (54). The peripheral concentration of IL-8 correlates positively with the severity of negative symptoms and the overall psychopathological presentation. The prognosis for negative symptoms is lower for patients with increased IL-8 levels. It appears that IL-8 levels go up as the disease progresses (16).

Interleukin 10 (IL-10)

Despite the fact that one meta-analysis indicated higher levels of IL-10 in FEDN patients (11), two later meta-analyses utilizing larger sample sizes demonstrated that its levels in this group were unaltered relative to healthy controls (35, 36). Intriguingly, relapsed patients with schizophrenia appear to have decreased peripheral IL-10 levels than healthy controls, but these findings are based on two small-scale studies (11, 21). One meta-analysis examined IL-10 peripheral levels in schizophrenia patients, revealing that these levels were unaffected (11). Similarly, peripheral IL-10 levels appear constant relative to healthy controls in people with clinically high and very high risk (37, 55). Peripheral levels of IL-10 have been reported to have positive relationships with the severity of negative symptoms, general psychopathological presentation, attention deficits, and the incidence of aggressive behaviors. On the other hand, researchers have discovered that low levels of IL-10 in the periphery are negatively correlated with cognitive deficits (16). Increased peripheral IL-10 levels were related to loss of microstructural white matter integrity in schizophrenia, supporting the notion that inflammation may play a crucial role in the pathophysiology of microstructural white matter in schizophrenia (34).

Interleukin 12 (IL-12)

Several studies identified higher levels of IL-12, one of the other T-helper 2 cytokines, in FEP patients, regardless of whether they were drug-naive or not, and in stable chronic patients, undergoing an acute relapse or recovering from one (11, 21, 56). In contrast to the studies that found elevated levels of IL-12, other studies were unable to duplicate these findings. They reported no change in FEAP levels of this cytokine (20, 45) and chronic antipsychotic-treated patients (53, 57). Cognitive deficits appear to be correlated with peripheral IL-12 concentrations (16).

Interleukin (IL-17)

A study that discovered a higher level of Th17 cell activation in people with recent onset schizophrenia has strengthened the idea that a discrepancy in the IL-17 pathway plays a role in schizophrenia (58). In schizophrenia patients, lower IL-17 levels were observed and a significant decline in Th17 cells (59). There were no changes in peripheral IL-17 levels between patients with FEDN and healthy controls (11, 60). Higher peripheral levels in FEDN were found in the largest sample (36). Studies have shown a negative association between IL-17 and negative symptoms. In contrast, there is a positive correlation between IL-17 and the severity of positive symptoms, the overall psychopathological presentation, and the frequency of aggressive behaviors (16, 45).

Interleukin 18 (IL-18)

IL-18, a pro-inflammatory cytokine that belongs to the IL-1 family, is secreted by macrophages, dendritic cells, astrocytes, and other epithelial cell types (17) and it is crucial to the Th1 immune response (61). It stimulates the production of gamma interferon IFN-γ in Th1 cells in synergism with IL-12 (62). Goldsmith et al. investigated IL-18 peripheral levels in psychosis, using an entirely FEDN sample and found that level was unchanged compared to healthy controls (11). Tanaka et al. identified increased levels of IL-18 in the serum of schizophrenic patients relative to healthy controls (61). Xiu et al. reported considerably more significant levels of IL-18 in the serum of patients with chronic schizophrenia compared to those with first-episode schizophrenia and healthy controls (63). In addition, significant positive relationships were established between IL-18 and the PANSS score in chronic patients. In FEAP patients, however, there was no significant link between IL-18 and psychopathology. Among chronic patients, there were no differences in IL-18 levels between those medicated with conventional and atypical antipsychotics, independent of treatment dose or duration (63).

Tumor necrosis factor (TNF-α)

TNF-α is one of the proinflammatory cytokines, and it is produced almost exclusively by macrophages. TNF-α boosts the cytotoxicity of macrophages, as well as the immune system's ability to create oxidative stress. Other cytokines, like IL-1, IL-6, and IFN-γ, are secreted in response to TNF-α. TNF-α is also produced by microglia and may change neuroplasticity and decrease neurogenesis by promoting the death of hippocampus stem cells (17). Multiple studies reported elevated levels of TNF-α, in FEDN patients (19, 21), FEAP patients (11, 64), and chronically ill patients taking antipsychotics, regardless of their acute relapse status (21, 22). It has been found that stable chronic patients receiving atypical antipsychotics had greater levels of TNF-α (65). In contrast, Miller at al. indicated that TNF-α levels in the population with schizophrenia were unaffected (21). FEDN patients with an illness duration of less than two years (22) and chronic patients with a disease duration of more than five years who take antipsychotics have decreased levels of TNF-α (66, 67). A lower TNF-α concentration in chronic patients who use antipsychotics is associated with positive symptoms and higher scores on the PANSS as well as with worse cognitive abilities. At the same time, this correlation was not found in FEDN. Positive

association exists between higher TNF-α levels and negative symptoms (16).

Interferon gamma (IFN-γ)

Interferon (IFN- γ) is a pro-inflammatory cytokine produced by activated CD4 T helper type 1 (Th1) cells, CD8 cytotoxic T cells, T cells, and natural killer (NK) cells, as well as, to a lesser extent, natural killer T cells (NKT), B cells, and professional antigen-presenting cells (APCs) (17). Several researchers revealed no significant changes in the levels of IFN-γ in FEDN patients (59, 68–70), but others found elevated levels of IFN-γ (21, 71). In contrast, one study discovered lower levels of IFN-γ in FEDN patients (72). Studies revealed higher levels of this cytokine in FEP patients, most of whom had used antipsychotics in the past (11, 20). Several investigations, including several meta-analyses, reported higher levels of IFN-γ in stable or acutely relapsing chronic schizophrenia patients (11, 21, 53). Reduced levels of IFN-γ were identified in individuals with acute psychotic symptoms who had not taken any drugs for at least six months, as well as in patients with chronic illness (73). No alterations of IFN-γ levels were found in clinically high-risk or ultra-high risk populations (37, 55).

Conclusion

In an attempt to find an inflammatory biomarker for schizophrenia, researchers try to separate different types of deviation from healthy controls in trait markers, which are related to hereditary and neurodevelopmental factors, and state markers related to the disease itself and its symptoms. Meta-studies in this field have come up with contradictory results. This may be due to small sample size, diverse patients` characteristics, heterogeneous population, different sampling methods and the difference between plasma, serum or whole blood cytokine profiles. The population with first-episode psychosis provides a unique opportunity to study the biochemical, clinical, and functional outcomes of psychotic disorders. Antipsychotic medication, comorbidity, and chronicity are all confounding factors that can be avoided with longitudinal study beginning at the onset of illness.

Establishing biomarkers as promptly as feasible after acquiring a disease can prevent early illness, increasing the prognosis. Early intervention reduces disease development and improves treatment results. Drug naïve FEP population has become an increasing research focus, with large-scale studies being done in both the United States and Europe.

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Shizofrenija, multisistemski poremećaj sa nejasnom etiologijom, povezana je i sa imunološkom disfunkcijom, uključujući i izmenjene nivoe citokina. Potencijalna uloga inflamacije u razvoju psihijatrijskih bolesti predmet je sve opsežnijeg istraživanja u poslednje dve decenije. Veza između inflamacije i shizofrenije prethodno je sugerisana zbog već utvrđene abnormalne proizvodnje citokina, njihovih odstupanja u koncentraciji i njihovim receptorima u krvi i cerebrospinalnoj tečnosti kod osoba koje boluju od shizofrenije. Patofiziološki mehanizam inflamacije kod psihoza još uvek je nejasan, te su istraživanja dala kontradiktorne rezultate. Populacija koja boluje od shizofrenije opsežno je istražena. Ipak, grupa bolesnika sa prvom psihotičnom epizodom pruža jedinstvenu šansu u proceni bioloških, kliničkih i funkcionalnih posledica psihoze. Rezultati koji se tiču koncentracije citokina su nedosledni, što je posledica različite metodologije istraživanja. Međutim, ustanovljeno je da postoji veza između markera inflamacije i simptoma bolesti. Razvoj biomarkera na početku ili u ranoj fazi bolesti pruža mogućnost za ranu prevenciju bolesti, što sa sobom nosi bolju prognozu. Intervencije u ranoj fazi usporavaju napredovanje bolesti i poboljšavaju ishod lečenja. Populacija sa prvom psihotičnom epizodom, koja nije tretirana medikamentima, dobija sve veću pažnju u istraživanju. *Acta Medica Medianae 2023;62(2):52-60.*

Ključne reči: *psihoza, inflamacija, shizofrenija, biomarkeri*

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Review article

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THE DEVELOPMENTAL PATH OF QUININE: WHAT CAN WE LEARN FROM HISTORY?

Aleksandar Jovanović[1,2], Dušanka Krajnović[2]

Almost 200 years have passed since the pure substance was first isolated, but scientists still face similar challenges. Quinine—the first chemotherapeutic agent in the treatment of malaria—is one of the good examples from history that testifies to the challenges in drug development.

The aim of the paper was to present the history of the discovery and synthesis of quinine and its importance in medicine.

Descriptive research was conducted using secondary data sources during September and October 2022.

Quinine is one of the first active substance whose effectiveness has been proven in clinical research. Its widespread consumption soon led to a shortage of quinine, and new sources of this valuable active substance had to be provided. The challenges of plantation cultivation were solved by developing botany and its chemical synthesis through organic chemistry. By researching quinine, numerous pharmacologically active substances such as caffeine and methylene blue were found, which would start a revolution in the chemical industry and the industry of organic synthesis. With the development of resistance to antimalarials, quinine experienced its heyday again because it proved to be effective even in resistant strains.

Quinine represents a significant historical discovery that influenced the development of many scientific disciplines, primarily pharmacy, medicine, and organic chemistry. The history of quinine provides us with an important historical lesson that we need to be aware of in today's time when the pharmacy is facing the significant challengesof developing new drugs. *Acta Medica Medianae 2023;62(2): 61-70.*

Key words: *quinine, history of pharmacy, drug development, antimalarial drugs*

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Introduction

The era of isolating pure active substances from plants started more than 200 years ago. More precisely, it was started in 1804 by a 23-year-old apothecary's apprentice Friedrich Willhelm Sertürner (1783–1841). He isolated the first pharmacologically active substance from opium obtained by cutting seed pods of the poppy, *Papaver somniferum* calling it morphine after Morpheus, the Greek god of dreams (1–4). Such and similar discoveries resulted in precise dosing and, therefore, in achieving greater efficiency in treatment, representing a type of revolution in medicine. With pure active substances, clinical experiments that could investigate the effectiveness of treatment on a quantitative basis are rapidly developing. Life expectancy in most of the world has increased from about 40 years earlier in the 20th century to more than 77 years today (5).

Some of the most significant isolations of pharmacologically active substances are shown in Table 1, created according to the paper of the author Prarojčić D. et al. (6). The discovery of synthetic drugs followed a little later, at the beginning of the 20th century. Some of the major therapeutic discoveries were the synthesis of barbitone (Veronal)—the first "true" hypnotic compound in 1903 (7) and arsphenamine (Salvarsan)—the first effective treatment for syphilis in 1910 (8); and the isolation of thyroxine in 1919 and insulin in 1922. One of the main discoveries was in 1928 when Alexander Fleming observed the antibacterial action of the mould *Penicillium notatum* (9).

Table 1. The most significant isolations of pharmacologically active substances

A year of isolation	The name of the active substance
1804	Morphine
1817	Nicotine
1818	Strychnine
1819	Brucine
1820	Colchicine
1833	Atropine Aconitine
1848	Papaverine
1890	Scopolamine

In the past, scientists generally came to the development of new drugs in two ways: accidental discoveries where the creativity, intuition, and meticulousness of researchers were of crucial importance (10, 11), while the other was the observation of specific side effects of the drug and their translation into positive therapeutic effects (3). Nowadays, when the pharmaceutical market is full of competition, there are unrealistically high expectations for discovering and producing "blockbuster drugs" (12). Most of the current difficulties in the pharmaceutical industry can be divided into two categories: the prevailing paradigm for drug discovery in large pharmaceutical industries and the limitations in identifying new compounds with the desired activity (13). Many large pharmaceutical companies have reduced their interest in using natural products in drug discovery screening, focusing on modern techniques (14, 15). However, natural products are still the only abundant sources of potential candidates ("lead" compounds) for developing new drugs (16). With new improvements in spectroscopy, analytical technologies and high-throughput screening, natural products can experience a renaissance in discovering new drugs (17).

The history of drug discovery and synthesis is full of stories of luck and perseverance. One such historical story is the quinine story, which actually begins with the history of plant usage. Quinine bark has long been used to treat malaria, an infectious disease that has caused perhaps even the most significant mortality in human history (18–20). The Peruvian Indians first discovered the effects of quinine bark on malarial fever, and after the colonization of Peru, the quinine bark reached Europe. Quinine bark soon received many names such as “cinchona bark”, "Peruvian bark", "Jesuit's powder", "cardinal's bark", "countess's powder" or "royal powder" (21).

Quinine bark extract was the first—and, for a long time, the only—effective medicine against fever, especially intermittent fever. The first notes of the use of quinine bark date back to the 17th century, after which the medical literature contains countless reports on the treatment of malaria using quinine bark and about a thousand specific studies on the subject (22). The use of quinine bark in England was founded by the London apothecary Sir Robert Talbor. He was making a secret, very successful preparation based on quinine bark. However, the secret was revealed in his posthumous book: *The English Remedy: or, Talbor's Wonderful Secret for Cureing of Agues and Feavers*. He combined Peruvian bark with rose leaves, lemon juice and wine (23).

Claims of its effectiveness often varied depending on the nationality of the practitioner as much as the quality (bitterness) of the bark (24). The high variability of the antimalarial effect of the quinine bark preparation was one of the main difficulties in using the bark. That variability came mainly from the type of Cinchona tree, the place where the bark was obtained, and several other variables that affected the active principle content of the bark. Franceso Torti was the first to conduct systematic studies on the effects of quinine bark on various types of fever in 1712 (21, 23).

Quinine bark was also used in the prevention of malaria, which is confirmed by the words of William Buchan, who, in 1781, wrote: "Take an ounce of the best Jesuits' bark, Virginian snake root, and orange peel, of each half an ounce; bruise them all together, and infuse for five or six days in a bottle of brandy, Holland gin, or any good spirit; afterwards pour off the clear liquor, and take a wine-glass of it twice or thrice a day". This may be the first recipe for gin and tonic

water (25). Quinine bark was used to combat malaria in soldiers during the siege of Belgrade in 1717 (26).

In addition to the treatment of malaria, quinine bark was also used in the treatment of ulcers, hemorrhoids, inflammation of the stomach, intermittent neuralgia, hemoptysis. The Pharmacopée universelle describes more than 100 official preparations based on quinine bark powder or bark extracts, which are believed to have special properties against a wide range of diseases, for example typhus or used as a tonic (21).

As a medicine that saved many lives, it had enormous importance and occupied the great attention of scientists of that time. In the era of great discoveries, when many other active substances from plants were discovered and isolated, quinine was also isolated—the active ingredient of the quinine bark. Quinine was isolated in 1820 by two French pharmacists, Pierre Joseph Pelletier and Joseph Caventou, after which quinine replaced the bark as the standard treatment for malaria (20, 27). By 1826, these chemists were producing over 3,500 kg of quinine sulfate each year, which can be considered the beginning of the modern pharmaceutical industry (28). Unlike other scientists, they made their process freely available, that is, they did not patent it (29). With the isolation of quinine, the first chemotherapeutic agent in the modern sense of the word was born (19, 21).

Therefore, this paper aims to present the history of the discovery and synthesis of the first drug used in malaria therapy and its importance for the development of pharmacy and medicine, to serve as a lesson about perseverance, effort, and hard work.

Methodology

We have conducted descriptive research in which mostly secondary data sources were used. The PubMed database and relevant websites were searched during September and October 2022. The following keywords were used during the search: "history", "cinchona", "quinine", "antimalarial drugs", and "malaria." After that, a selection of relevant works and websites was made. The next step was reading and analyzing the selected works. The final step was data synthesis.

Results and Discussion

Quinine—isolated active substance

With the pure quinine substance, the dosage becomes precise. It can be adjusted to the individual needs of patients, as a result of which the effectiveness of malaria treatment has increased many times. Since Pierre and Joseph were pharmacists with less experience, they left to more experienced doctors to prove the effectiveness of the newly isolated natural product. They soon confirmed its effectiveness, established its specificity in the therapy of occasional (malarial) fevers, and by the end of 1821, gave instructions for its use in *Formulaire pour la préparation et L'emploi de plusieurs nouveaux médicaments* (30, 31). First, its safety was tested on dogs, and when it was defined as safe for use in therapeutic doses, it was tested on hospital patients. The results were as expected—quinine achieved remarkable efficacy, and numerous medical observations and case reports from around the world soon pointed to its specificity for "malarial" fevers. However, isolated quinine had a much higher price than the quinine bark, so the question arose: is it profitable to use quinine instead of cheaper crude extracts of the bark? Soon scientists come to a very simple conclusion: quinine or its salts should be used instead of bark extract to treat intermittent fevers (21).

The dose of quinine that was used for therapeutic purposes was adjusted to the dose of quinine bark that was previously used, which led to significant variability in dosage. Most doctors gave their patients 3–5 grains (approximately 0.2–0.3 grams) of quinine no more than 2 times a day, while some administered 5 grains every 6 hours. Italian physicians have reported using up to 25 grains daily to treat severe and relapsing intermittent fevers. Despite these variations and uncertainties, a consensus has been reached on treatment: apply 5 to 15 grains of quinine daily, divided into several doses. Discussions about the method of application and dosage of quinine continue to this day. Warrington Yorke best described these methods. He stated that “The use of quinine has been known for 300 or 400 years, yet no one is even now able to state how the quinine should be taken, in what manner, and in what doses” (21, 32, 33).

Following the hypothesis that quinine is found in the coffee tree (because it belongs to the same family as quinine), numerous studies followed, which resulted in the isolation of caffeine in 1821. Soon other alkaloids were isolated from the bark of the quinine tree: firstly quinidine, cinchonine, and cinchonidine, and an additional 25 alkaloids related to quinine were isolated by 1884, and 6 more between 1884 and 1941. Pasteur, a versatile French scientist, produced several "toxins" (cinchotoxin and quinotoxin—known initially as quinicine) from quinine, which would prove crucial 50 years later during the first attempt to synthesize quinine, and their role is still prominent in today's science (30).

With the colonization of malarial parts of the world, the demand for quinine became even more remarkable, and the supply of herbal drugs from South America became insufficient (23, 34). In the mid-19th century, bark and pure quinine were always in short supply as they were the only known effective treatment against malaria. This initiated ideas of plantation, cultivation, and

synthesis of quinine, as the only possible alternatives to ensure a continuous and abundant supply of quinine (23, 30).

Cultivation of cinchona tree

Although the plantation cultivation of cinchona tree looks very simple at first glance, at the time, it was a big challenge. In the beginning, numerous expeditions were organized in search of trees, seedlings, and seeds of quinine, but the lack of botanical knowledge made the cultivation unsuccessful (37). At that time, quinine was cultivated in Ecuador, Peru and Bolivia, and the export of quinine seeds was strictly prohibited. England took the lead in these expeditions, and the entrepreneur Charles Ledger, in 1865, finally got hold of several kilograms of high-producing quinine plant seeds and began its cultivation. The seedlings were grown at Kew Gardens from where they were to be distributed to where they would be planted (21, 25).

The first cinchona plantation established outside the American continent was in Ceylon and India, and the quantity of the drug was sufficient to meet the needs of the English colonial army (30, 35). However, the Dutch soon set up a plantation in Java thanks to Charles, who sells them cheap seeds, because the English government was not interested in buying them (25, 30, 36). Due to the favorable climatic conditions, these plantations became the primary sources of quinine bark and the Netherlands took control of the world trade in quinine, with more than two-thirds of the world's share, until the Second World War, when Japanese control of Java forced the Allies to look for alternative supplies and synthetic substitutes for quinine. Until 1913, quinine bark was characterized by low prices, but that year the "Quinine Agreement" came into force, which established a specific, higher price for the bark, thus creating the first pharmaceutical cartel in the world (37, 38). Figure 1 taken from a 2013 paper by Goss A. shows workers on a cinchona plantation in Java (39).

During the period of British colonization of India and other tropical countries, in the early 19th century, medicinal quinine was recommended to British officials and soldiers as a prophylactic against malaria, where it was mixed with soda and sugar to mask its bitter taste, forming tonic water (40).

Figure 1. Workers on the Cinchona plantation Ramawatie, West Java. Collection Tropenmuseum, Amsterdam, coll. nr. 10012774. Taken from reference 39

Synthesis of quinine

However, this second strategy proved to be a much more demanding task. It will take almost a century to be realized. Nevertheless, the synthesis of quinine would bring about a scientific revolution and play an important historical role in organic and pharmaceutical chemistry (30).

The first to speak of the challenge of its synthesis was August Wilhelm von Hofmann, then director of the Royal College of Chemistry in London, who in 1849, declared his intention to synthesize the lucrative quinine to demonstrate the ability of organic chemistry to solve social needs. Shortly after that announcement, the race for synthetic quinine heated up. The French Pharmaceutical Association invites chemists by offering a prize of 4,000 francs to one who first synthesizes quinine. No one claimed this award (30).

Chemical synthesis was in its infancy at the time. Scientific research in this area has often been done by trial and error based on intuition. Moreover, there were no related concepts for the structure of compounds (these ideas appeared a decade later with the development of structural theory). The molecular formula of quinine postulated by Hoffmann ($C_{20}H_{22}N_2O_2$) had two hydrogen atoms less than the correct formula ($C_{20}H_{24}N_2O_2$) established by Adolf Strecker in 1854. This knowledge prompted the beginning of the experimental phase of Hoffmann's project. Soon, however, economic support begins to wane due to the impatience of wealthy sponsors who

worry about the lack of results from their investments and begin vehemently debating the true virtues of applied organic chemistry and its ability to produce something worthwhile (41).

During the Easter holidays of 1856, knowing the exact molecular formula of quinine and following the ideas of his mentor Hoffmann, eighteen-year-old William Henry Perkin decided to "reproduce" quinine (42). However, the experiments were mostly unsuccessful. In one of his many experiments, he accidentally discovered the first synthetic aniline purple dye called "mauve", which launched the synthetic dye industry and the birth of the modern chemical and organic synthesis industries (43–45). Perkin developed processes for the mass production of paint and, in 1857, with the financial support of his father, opened a factory near London to commercialize his discovery. It was the world's first large organic chemical factory, the beginning of the aniline dye industry, which itself was the initial driving force in the global chemical industry (19, 30, 32).

Paul Ehrlich soon discovered that methylene blue was particularly effective in staining malaria parasites. Under this assumption, the dye had a toxic effect on parasites. Together with Guttmann, he used this drug in 1891 to treat malaria. Methylene blue therapy proved successful and became the first synthetic drug ever used in therapy (46, 47). Methylene blue also contributed to the development of many antipsychotics and antidepressants that were discovered after substitution of side groups on the methylene blue scaffold (48).

In the next 50 years, there were no serious attempts at synthesis. However, structural theories were developed in that period, and organic chemists realized that the structure of quinine is more complex than previously thought and that elucidating the structure was the first cork in a rational approach to synthesis. It was a great challenge for the chemists of that era and required the application and combination of all previous knowledge in chemistry. A breakthrough occurred in 1908 when Paul Rabe theorized the correct chemical structure of quinine, but some stereochemical questions remained unresolved, and research continued (30, 49).

World turmoil during the First World War made it impossible for Germany to supply quinine from Java. As a result, German soldiers who fought in southern Europe suffered greatly from malaria. In order to prevent this event from happening again, German pharmaceutical chemists were engaged in the 1920s to find a synthetic alternative to quinine, which they succeeded in doing. The first effective aminoquinoline drug in the treatment of malaria was pamaquine, synthesized in 1926 (50). By 1932, they had synthesized quinacrine, also known as mepacrine (Atabrine®), a simplified version of quinine (51). This drug became the main drug used in the treatment of malaria during the Allied war operations in the Pacific, despite the fact that the skin of its users became yellow (52). Its use was enforced by giving it at mealtimes and warnings about the danger of not taking it. One sign posted at a hospital in Papua New Guinea had two human skulls mounted on a bulletin board that read "These men did not take their Atabrine" (40).

While the search for the structure was still going on, Rabe and Kindler, in 1918, took the first big step towards synthesizing quinine since Perkin's famous "failure" by presenting a synthetic sequence for obtaining quinine and quinidine from quinotoxin. After countless experiments, assembling scientific results like a mosaic, in 1931, Paul Rabe determined the final stereochemical structure of quinine, thereby opening the door wide to its synthesis (30).

The Second World War brought similar problems. America was left without quinine due to the Japanese conquest of the Javanese territory. Therefore, the mepacrine trade between the USA and Germany stopped. American soldiers who fought in the Pacific ran out of malaria medicine and fell victim to it (53). Although they soon developed their synthesis of mepacrine, Americans fell under the influence of Japanese propaganda called "Tokyo Rose," in which the Japanese spread the lie that mepacrine causes sterility. Many Americans stopped using it and fell victim to malaria. This led to the development of new antimalarials such as santoquine and chloroquine, but also to the investment of significant additional efforts for the total synthesis of quinine. Chloroquine was first synthesized in 1934 by H. Andersag, an industrial chemist of the Bayer Company, under the name Resochin®. After synthesis, its effects on birds infected with malaria were tested, where it was determined that it was as effective as Atabrine, but more toxic. This led Bayer to abandon the development of this drug. However, the development of chloroquine in the USA began in 1943, and it was evaluated on dogs and monkeys, with the latter study showing that it was four times less toxic than sontaquin. After that, in 1946 it was chosen as the drug of choice for the treatment of malaria (23, 54). Santoquin was synthesized by Bayer industrial chemists in 1936 as an attempt to develop a less toxic analogue of chloroquine (55). In 1946, primaquine was synthesized in America, replacing the side chain terminal NEt2 group with an NH2 group, in order to reduce the toxicity of pamaquine (56).

Finally, in 1944, Robert B. Woodward and William von Eggers Doering at Harvard University reported the first total synthesis of quinine—the most famous cinchona alkaloid claimed to be "the drug that has alleviated human suffering more than any other in history". In Figure 2, taken from a paper by Ball published in 2008, we can see Robert Woodward and William Doering. That was the end of the almost 100-year era of man's attempt to master this unique product of nature.

This discovery led to Robert and William being hailed as heroes (32). However, there was

one problem. They succeeded in synthesizing hinotoxin, and Rabe and Kindler took over the further procedure of its translation into quinine, but they failed to repeat it. That is why research into the synthesis of quinine continues. In 1970, Milan Uskoković and co-workers discovered the first real total synthesis of quinine. Another great success in this field was achieved in 2001 when Gilbert Stork published the first total stereoselective synthesis of quinine (57, 58).

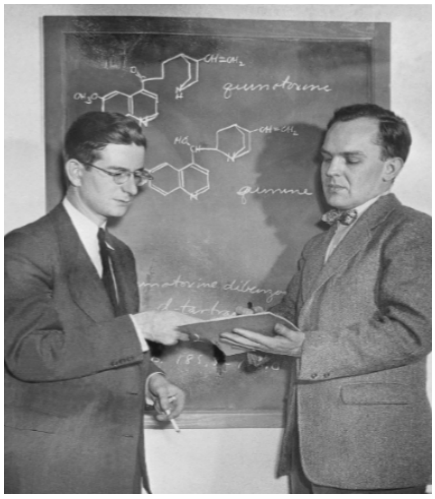

Figure 2. Robert Woodward (left) and William Doering (right). Figure taken from reference (32)

Quinine therapy

Quinine is a short-acting drug with an extremely bitter taste. In therapeutic doses, it often causes a series of unpleasant symptoms known as cinchonism that include tinnitus, dizziness, headache, dysphoria, nausea and vomiting. Nevertheless, the availability of quinine as a pure active substance allowed it to be widely used in the prophylaxis of malaria, especially among soldiers. However, even in frequent repeated doses that can cause cinchonism, quinine does not actually prevent infection (59). That is why the soldiers avoided using it. In Figure 3, taken from the work of the author Eiden published in 1998, we see propaganda material urging soldiers to use quinine: Soldier, take your quinine daily (60).

Figure 3. Propaganda material urging soldiers to use quinine. (E. Chast Collection, Paris. From the exhibition catalogue "De l'élixir au génie génétique—deux siècles de sciences pharmaceutiques hospitalières", Paris 1995). Figure taken from reference (60)

The famous "Koch's method" of quinine application states that the widespread use of quinine would not only cure individuals but, if used by all infected persons, would break the cycle of re-infection and could lead to the elimination of malaria. This method was encouraged by the Governments of certain countries, which led to a new expansion of the use of quinine (39).

Quinine was one of the first drugs produced by the world's pharmaceutical industry. Factories

processed quinine bark, extracting the alkaloid quinine, converting it into quinine sulfate, which were suitable for medical use. The doses of quinine that were established immediately after the discovery of quinine have not been changed for years. Unlike the dose, the length of therapy varied from a few days to a few months. Before the acute phase of malaria, 1 g of quinine was administered, followed by smaller daily doses (0.25–0.4 g) over a longer period of time. However, the discussions about dozing of quinine did not stop. This later led to changes in the treatment protocol. Very high doses of quinine have been used in fevers that did not respond to lower doses. However, those high doses led to very toxic effects (21, 39).

Until the availability of effective synthetic antimalarial drugs in the late 1940s, quinine was the only reliable antimalarial drug. Because of its side effects, its use as a first-line treatment is limited, but it is still valuable as an adjunct to other treatments and can save lives. After the development of other antimalarial drugs, quinine was briefly neglected. However, with the development of resistance to antimalarials, quinine experienced its heyday again because it proved to be effective even in resistant strains (61). As of 2006, WHO no longer recommends quinine as a first-line drug for malaria.The 2010 WHO guidelines recommend a combination of quinine and doxycycline, tetracycline, or clindamycin as the second-line treatment for uncomplicated malaria, and a combination of quinine and clindamycin for the treatment of malaria in the first trimester of pregnancy (18).

Quinine continues to play a significant role in the management of malaria in sub-Saharan Africa and other malaria endemic areas. For example, in Uganda, quinine is prescribed for up to 90% of children <5 years of age with uncomplicated malaria (62).

By 2009, 31 African countries recommended quinine as a second-line treatment for uncomplicated malaria, 38 as a first-line treatment for severe malaria, and 32 for the treatment of malaria in the first trimester of pregnancy (63). There is no doubt that quinine will continue to play an essential role in human history soon (18).

Conclusion

Quinine represents a significant historical discovery that influenced the development of many scientific disciplines, primarily pharmacy, medicine, and organic chemistry. In addition, quinine, as the first effective antimalarial drug, saved many lives and significantly contributed to the public health of that era. However, the challenges faced by scientists were significant. Although they had well-established hypotheses, in order to prove them, particular prerequisites, such as advances in analytical techniques and chemical theories, had to be developed. Scientists followed their intuition even when everything pointed to them being wrong. However, patience, meticulousness, and persistence made them prove their hypotheses and make significant discoveries. From this, we should learn an important historical lesson by emulating the scientists of that time because today's scientists are also facing similar challenges.

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Declaration of competing interest

None declared.

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RAZVOJNI PUT HININA – ŠTA MOŽEMO NAUČITI IZ ISTORIJE

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Prošlo je skoro više od 200 godina od prvog izolovanja čiste supstance, ali se naučnici i dalje suočavaju sa sličnim izazovima. Hinin, prvi hemoterapeutik u terapiji malarije, jedan je od dobrih primera iz istorije koji svedoči o izazovima u razvoju lekova.

Cilj ovog rada bilo je prikazivanje istorije otkrića i sinteze hinina i njegovog značaja za razvoj farmacije i medicine.

Sprovedeno je deskriptivno istraživanje korišćenjem sekundarnih izvora podataka tokom septembra i oktobra 2022. godine.

Hinin je jedan od prvih lekova čija je efikasnost dokazana u kliničkim istraživanjima. Široka potrošnja ubrzo je dovela do deficita hinina, te su morali da se obezbede novi izvori ove dragocene aktivne supstance. Izazove plantažnog gajenja rešio je razvoj botanike, a izazove hemijske sinteze razvoj organske hemije. Prilikom istraživanja hinina pronađene su brojne farmakološki aktivne supstance, poput kofeina i metilensko plavog, što je pokrenulo revoluciju hemijske industrije i industrije organske sinteze. Sa razvojem rezistencije na antimalarike, hinin ponovo doživljava svoj procvat, jer se pokazao efikasnim i kod rezistentnih sojeva.

Hinin predstavlja značajno istorijsko otkriće, koje je uticalo na razvoj mnogih naučnih disciplina – farmacije, medicine i organske hemije, pre svega. Istorija hinina pruža značajnu istorijsku lekciju, koje treba da budemo svesni u današnje vreme, kada se farmacija suočava sa velikim izazovima razvoja novih lekova. *Acta Medica Medianae 2023;62(2):61-70.*

Ključne reči: *hinin, istorija farmacije, razvoj leka, antimalarici*

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THE IMPORTANCE AND ROLE OF HEALTH WORKERS IN THE RELATIONSHIP WITH THE PUBLIC IN THE FUNCTION OF RAISING AWARENESS ABOUT ONCOLOGICAL DISEASES

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Public relations represent a management function and should establish mutual quality relations between the health institution and the public, on which the success of the organization depends. Public relation is a complex communication process, which includes the activities of a healthcare organization, aimed at developing cooperation with target groups, which can be internal and external. Good communication contributes to doctors and medical staff maintaining their professionalism at a high level, while following the principles of medical ethics, which greatly improves the quality of life of oncology patients. Communication is a key element of the patient's trust in the medical staff, and it plays a vital role in the treatment of oncology patients.

Dialogue through which support is provided is an extremely valuable resource and can be the most important (sometimes the only) element of patient care. The basic idea of an effective therapeutic dialogue is that the patient should get the impression that someone has heard and understood his fears and worries, in which the nurse's role is essential because she spends most of her time with the patient. It may happen that some of the problems can be solved, that some emotional moments can be overcome or that some needs can be satisfied, but even when there is no solution, the simple act of dialogue will reduce the patient's discomfort. *Acta Medica Medianae 2023;62(2): 71-76.*

Key words: *public relations, communication, oncologic diseases*

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Introduction

In modern working conditions, an organization (company, client) is surrounded by a large number of service users and has a large number of public opinions with whom it needs to build contacts and communication. Employees in the public relations sector should therefore identify the target group and the public and build long-term relationships of mutual understanding and respect.

With real and honest communication, sympathy is gained, as well as trust, and a relationship of mutual understanding and acceptance is established. In this way, communication with the target public acquires and becomes an essential element of identity.

The reputation of an organization depends on how well it is known to the public and to what extent the public supports its goals. That is why the management of organizations increasingly relies on public relations experts. The job of an expert (PR manager) is to represent the organization, maintain good relations with the public and raise the effects and image of the company.

Public relations in healthcare

Public relations are indispensable for the functioning of a health institution. That is why the role of the PR manager in these institutions is indispensable, in internal and external communication, organizing events, presentations, creating the institution's image.

Since healthcare is a discipline to which the population is very sensitive and where quick and

accurate information is needed, it is clear that public relations are of extreme importance.

Public relations are conscious, planned and constant efforts to achieve and maintain good relations and mutual understanding between the work organization and the public. The practice of public relations is a social activity that deals with the analysis of trends and developments, predicting their possible consequences, advising the management of the organization and implementing planned programs of activities that serve the interests of both the organization and society.

Every work organization, including health-care, must clearly set public relations goals and each goal requires a separate strategy. If we want the goals set in communication projects to be measurable, certain conditions must be met:

- Clearly define the target group
- Determine the nature of the desired change
- Have a clear attitude about what we want to achieve
- Determine the scope and size of the changes
- Determine the time by which we want the change to happen

The public as an area of free communication includes all individuals or groups of people with whom an organization communicates or wants to communicate. For the development of an efficient health system, communication is crucial, because it is an integral part of the health service.

Modern operations of health institutions cannot be imagined without communication with the environment. Through communication, the institution informs the public about its services, possibilities for meeting the needs of patients, and in this way creates a positive image of itself. Public relations must be aimed at creating and building trust between the public and the healthcare institution.

The role of public relations in healthcare

The health system is one of the most important and complex systems of a society so the role of management is extremely responsible and demanding. PR therefore has the role of creating a climate suitable for the work of both patients and employees and achieving good results in the business of a certain health institution.

“Advertisers have the task of selling the audience a performance about itself, with the advertiser's product being integrated into that performance. The task of the media is to skillfully sneak into the general social life... in which they achieve significant success” (1). Therefore, PR needs to know the media well, all their possibilities, and find the best way to present their company and what it offers in them in order to reach their users in the best way.

“When we study content, we study, in fact, the way in which ideas and images are expressed and represented. This phenomenon is sometimes called encoding, or the production of symbols and symbol systems... The study of representation and the representation of human affairs is actually the study of grounded non-deterministic systems. There are a certain number of ways to represent an object, event, or action - one can always make a different original sketch - but each of these ways is grounded in the goal of focusing on what is represented, the person/or medium performing the representation, and the audience to whom the representation is intended” (2).

A good PR must know the market and the advertising system well. Advertising is "embedded in the very foundation of commercial mass media, because it enables the production and distribution of information and entertainment for a wide range of population, at a very low cost per consumer... In commerce, advertising is increasingly becoming a means by which manufacturers launch their products and maintain sales levels. The advertiser creates a need, and then convinces us that the product fulfills that need. Advertising, too, bypasses the product's effect by bragging about, enough to make it itself a reality” (3).

The attitude towards users has also been significantly changed, so it is moving from the consumer mass to target groups. The population is divided into meaningful units, so messages are created for those segments - target groups, by which we are attracted. “This means that analyzing with which value group a person identifies with, we can predict to a considerable extent which products and services he wants” (4).

The main reason why organizations engage in advertising is to better sell their special brand, e.g. some health institutions deal with advertising in order to increase the proportion of people who use the services of a particular health institution.

Thus, PR should achieve cooperation with the public, research the attitudes and needs of a certain target group, organize public appearances, plan and order advertisements, organize communication with the environment. Healthcare institutions themselves are under increasing pressure and public expectations.

That is why PR has the responsible task of developing and strengthening general understanding, trust and sympathy towards the organization, influencing public opinion in the interest of the organization, understanding and cooperation of all interested parties within the framework of a common goal, supporting general interests.

The PR is expected to select information based on relevance, communicators (transmitters of information) and translation of the language of the profession into a language understandable to every individual. Another important role of PR in the health sector relates to the education of citizens on a wide range of important health

topics, starting from topics related to legal health regulations, to the consequences and effects of health behaviors and habits, including symptoms of mass health problems. Their effectiveness depends on whether they know how to market it in the right way, at the right time and in the right place.

Public and oncologic diseases

Every year in Serbia, about 36,000 people get sick from cancer, and more than 20,000 lives are lost. One child falls ill in our country every day from one of the malignant diseases. Compared to others, Serbian citizens die from this disease in greater numbers due to, as doctors say, bad lifestyle habits, failure to attend regular preventive examinations, but also the lack of appropriate therapy and treatment.

In Serbia, fewer people get cancer than in some other countries, but the mortality rate is much higher. Guided by these statistics, it seems we are wrong in many things, and we must urgently react and correct it. The level of awareness about the prevention of malignant diseases (both among citizens and among medical personnel) must be at a much higher level. We need to correct life habits, raise to a higher level prevention as well as the knowledge of our doctors. In that way, we would come up with some new drugs and treatment techniques.

Pr activities in the role of helping oncologic patients

PR activities in the role of helping oncological patients can, and should, be of great importance, impact and help to people suffering from oncological diseases.

One of the good ways is to organize forums, in the spirit of multidisciplinarity, where all modalities of modern oncological treatment would be represented, with the patient at the center of every consultative decision and therapeutic plan. The focus is on the importance of specialized oncology centers, the adequate diagnosis, treatment and follow-up of oncology patients, all with the aim of achieving a better treatment outcome.

In the framework of carefully selected sessions, the novelties would be introduced to the public in the treatment of primary and metastatic cancer, the specifics of personalized radiotherapy and the current problems of medical oncology.

It is advisable to hire experts for these diagnosis needs to present contemporary views on, treatment and follow-up of oncology patients, with special reference to indications, radiological and therapeutic treatment protocols.

In addition to such forums, PR activities in the role of helping oncology patients can also include symposiums for nurses and technicians of oncology institutes, as well as organizing forums for oncology patients.

Communication with oncologic patients

Good communication is a key element of the patient's trust in the medical staff, and plays a major role in the treatment of oncology patients. Communication is an important but neglected factor that affects the quality of treatment in oncology. Poor communication is a result of inadequate training of the medical staff for the conversation who is often too sensitive and emotional, as well as the environment in which the conversation takes place.

The fundamental principles of effective communication with oncology patients are based on an approach in which the patient is the center of attention, which implies respect for the wishes and needs of patients and their families. Such communication has three basic values: it takes into consideration the patient's needs, perspectives and individual experiences, gives him the opportunity to participate in the treatment, and finally improves the relationship between the patient and the medical staff.

Communication where the patient is the center of attention is characterized by verbal and non-verbal behavior that should lead to the discovery, understanding and evaluation of the patient's perspective (e.g., concerns, feelings, expectations).

At the Institute of Oncology and Radiology of Serbia, the Serbian Association for Psycho-Oncology "SAPO" was founded (similar to associations in the region and in developed countries of the EU and the world), with the aim of providing psychological support, as a good form of communication, to people of different ages, patients with malignant diseases, as well as family members of patients, during all phases of demanding oncological treatment.

Information and communication Messages

It is often found in the literature that the term communication is associated with the term "information". The concept of communication is very closely related to the concept of information; therefore, some theorists point out the fact that information is the core of communication.

The term information originates from the Latin language and it means to form, to educate, that is, to present something. The word information etiologically refers to a number of key terms used in everyday language. In everyday speech, the term information is used much more often in the sense of the content of communication than in the original meaning as a term denoting the process of forming thought content (5).

The basic difference between communication and information can also be understood as the difference between communicative and informative activities. Communicative practice implies the creation and establishment of symbolic activity

as a concrete social relationship, while information can be understood as the content of communication or the subject of a message. (6)

In other words, communicative practice as a form of social relationship represents a social fact that dimensions but also limits the use value of any information. It can be pointed out that if information is understood as new knowledge than the message is actually shaped information.

In practical life, the term "information" has a very widespread use. It can function both by itself and as a substitute for other idioms such as data, news, notification. In the professional literature, there are over a hundred definitions of information, but not one of these definitions is universally accepted. With the help of information, we know everything that was available and understandable about the universe in which we live. Based on information, one tries to interpret what is factual from the past or to construct something and predict the future. However, information is not defined by the number of sounds, letters or electronic bits by which a slice of the universe is "measured" and mentally" accepted" (7).

In the world of men, signs and symbols, it is difficult to discover rules (if they exist), and there is even less lawfulness. Based on the use of signs and symbols, man tends to master both natural and spiritual processes and phenomena. It is best for man to establish what the information means. Only then will he decide whether he will react to her. It seems undeniable that human information must satisfy two more criteria: the criterion of utility and the criterion of probability (8).

Approach to the family of oncological patients

Psychosocial support is very important in the process of adaptation and further treatment of patients. The disease inevitably brings changes in lifestyle and goals, which directly affects the patient's family. When informing an adult about his illness and treatment, in most cases a member of the family or close environment is also present. In the case of minors, full information is given to parents or guardians, who at the same time give their consent to treatment. When we talk about persons deprived of business capacity, the information is given to their guardian. With the patient's permission, we talk to the closest family members. This is important for psychological support and the necessary care that must be provided at home between treatment cycles or in the terminal phase of the disease. It is usually said that the disease engages the whole family, but in some situations, it deepens misunderstandings and difficulties that existed before. It is important to overcome the stressful situation as soon as possible and to maintain communication in order to achieve a new balance that respects the new opportunities. The patient and family members should be offered professional psychological help and support (9)

A place that ensures privacy should be provided for the conversation. The prognosis should be discussed in a way that the patient understands avoiding too many medical details. During the interview, the patient and his family should be allowed to express their emotions. The patient should be informed about events in the family, work, events that are discussed in society.

Such communication will mean that the patient is not oriented only to thoughts about the disease, which means that the disease has not disabled him and excluded him from everyday life.

When talking with patients who are in the terminal phase of the disease, one should examine how much the patient and his family know about the disease and the outcome, what the patient's attitudes are and his possible reactions, reexamine one's own feelings, attitudes, compassion and regret.

The obligation of the medical staff is to inform the patient about the results of the analysis, diagnosis of malignant disease and treatment, which is the ethical obligation of the doctor, and he will assess how much information the patient can "mentally bear".

Informed consent implies the doctor's obligation to inform the patient about the method of treatment, whereby the patient is left with the freedom of choice and self-determination. The patient has the right to consult another doctor, he has the right to another professional opinion (10).

In the conversation with the patient, one should be active with expressed empathy, sensitivity, objectivity, flexibility and relative absence of serious emotional problems. The patient should be presented with the problems and the possibilities of solving them, check if he understood what was said, repeat it in a way that he can understand. After the hospital part of the treatment is completed, it is necessary to agree on the patient's controls and propose a specific plan for the future (individually created, with the recommendation of inclusion in a psychological-oncology counseling center, physical rehabilitation).

In addition to the method of treatment (chemotherapy, surgical treatment, types of cytostatic, radiotherapy), the patient should be informed about the immediate side effects of the treatment (nausea, alopecia, loss of appetite, vomiting) and the possible solution (nausea - antiemetics, alopecia - wig), and to be informed about the late possible side effects of the treatment (e.g. sterility) (11).

Obstacles to good communication with oncological patients

What is happening in practice is, unfortunately, the so-called three-minute medicine, which means that the lack of time to talk is the main reason for the lack of information and the

justification for inadequate communication, because most health workers have to work with a large number of patients. However, if time is spent on effective communication, time can be saved later, because the patient feels that you really hear and understand him, so a mutual understanding of the patient's situation is achieved, which makes the relationship of compassion more effective (12).

An obstacle to good communication can be the patient's inability to express feelings, the shame of admitting that they have a problem with overcoming their own situation, or if the professionals seem too busy to them. The problem can arise if health workers do not feel confident or believe that emotional support is not part of their professional role and that the patient's emotional response does not affect them personally.

The tendency to use behavior that blocks approach and avoids talking about difficult emotional topics is well known in professional literature. It is very important to remember that both healthcare professionals and patients can use avoidance tactics. However, we must emphasize that it is very important to avoid behavior that blocks communication, such as giving too much comfort without identifying the underlying problem. It is necessary to avoid downplaying mental pain as something normal, focusing exclusively on physical aspects, changing the subject or incidentally cheering up the patient (13).

Conclusion

People can be strongly affected by the way we communicate with them, whether it is verbal or non-verbal communication. The first contact and the way we start communicating with the interlocutor can be of crucial importance. It is very important, both with a healthy interlocutor in everyday life, and with a health care user with an existing or potential problem, how the first communication contact will be established, which can also have a "halo" effect.

Medical staff, PR managers, information services, in addition to technical skills, must also possess good communication skills, because in their work they constantly establish different relationships with the public, sick and patients, families, other health workers and organizations, and thus act indirectly between them.

The relationship that is established with a sick person is especially important, which can influence the development of the disease itself. That is why the old saying: "Disease licks the body and bites the personality", indicates the beneficial relationship of the healthcare worker towards the user of healthcare services. Words addressed to a sick person should not stamp the personality of the patient.

Often, the stereotypical medical "vocabulary" makes the patient and his personality completely equate with the disease, and the patient is often referred to as a "case". In any case, the patient is also a human being, and the language we use to address him must reflect respect and preserve his dignity. In every form of communication, we face a real challenge. We need to show sincere respect to the interlocutor, in order to help him express his feelings freely and openly.

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ZNAČAJ I ULOGA ZDRAVSTVENIH RADNIKA U ODNOSU SA JAVNOŠĆU U FINKCIJI PODIZANJA SVESTI O ONKOLOŠKIM BOLESTIMA

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Odnosi sa javnošću predstavljaju funkciju upravljanja i treba da uspostavljaju međusobno kvalitetne odnose između zdravstvene ustanove i javnosti, od čega zavisi i uspeh organizacije. Odnosi sa javnošću su složen komunikacijski proces, koji obuhvata aktivnosti zdravstvene organizacije usmerene na razvijanje saradnje sa ciljnim grupama, internim i eksternim. Dobra komunikacija doprinosi tome da lekari i medicinsko osoblje drže svoju profesionalnost na visokom nivou, uz pridržavanje principa medicinske etike, što u velikoj meri poboljšava kvalitet života onkoloških bolesnika. Komunikacija je ključan element poverenja bolesnika u medicinsko osoblje i igra veliku ulogu u lečenju onkoloških bolesnika.

Dijalog kroz koji se pruža podrška je izuzetno vredan resurs i može biti najvažniji (nekada jedini) element zbrinjavanja bolesnika. Osnovna ideja delotvornog terapeutskog dijaloga je da bolesnik treba da stekne utisak da je neko čuo i razumeo njegove strahove i brige; u tome je uloga medicinskih sestara velika, jer provode sa obolelima verovatno najviše vremena. Može se desiti da su neki od problema rešivi, da je neke emotivne momente moguće prevazići ili da je neke potrebe moguće zadovoljiti, ali, čak i onda kada nema rešenja, jednostavan čin dijaloga umanjiće nelagodnost bolesnika. *Acta Medica Medianae 2023;62(2):71-76.*

Ključne reči: *odnosi sa javnošću, komunikacija, onkološke bolesti*

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Case report **UDC: 616.36**
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CAROLI'S DISEASE: A DISEASE THAT RARELY COMES TO MIND: A CASE REPORT

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Caroli's disease is a rare disease characterized by dilatation of large intrahepatic bile ducts. It occurs in the classic form presented by repeated episodes of cholangitis (Caroli's disease), as well as in the form of syndrome with the development of fibrosis and cirrhosis of the liver (Caroli's syndrome). The disease can occur throughout entire life, but mostly before the age of 30. The incidence of this disease is estimated at about 1 in 1,000,000 cases for Caroli's disease and 1 in 100,000 for Caroli's syndrome. The main symptoms are: fever, jaundice, itchy skin, pain under the right costal arch, nausea and vomiting. Possible complications are the development of liver fibrosis and cirrhosis and cholangiocellular carcinoma. Diagnosis is made by clinical and ultrasound examination, computed tomography, more often by magnetic resonance cholangiopancreatography and liver biopsy. We present a clinical case of an elderly patient who has been suffering from Caroli's disease for a few years now. Diagnostic challenges and applied therapy are presented. *Acta Medica Medianae 2023;62(2): 77-82.*

Key words: *Caroli's disease, Caroli's syndrome, cholangitis, cholangiocarcinoma*

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Introduction

Caroli's disease is a rare disease characterized by dilatation of large intrahepatic bile ducts (1). The modern classification includes two forms of the disease: classical Caroli's disease and Caroli's syndrome. The classic form is isolated involvement of the bile ducts, while Caroli's syndrome is characterized by the development of periportal fibrosis and portal hypertension. The disease appears in more common diffuse form presented in both lobes of the liver, and significantly much less often in one, usually the left lobe (2).

The exact incidence of this rare disease is not accurately known, but it is estimated that classic Caroli's disease occurs in 1 in 1,000,000 cases, while Caroli's syndrome affects 1 in 100,000 people. It is difficult to identify all cases of Caroli's syndrome, because its characteristics can look like many similar conditions (3). It is estimated to affect men and women equally, with a slightly higher incidence of the Asian and the younger population (1, 4).

The disease is inherited in an autosomal recessive pattern. A mutation in the gene for polycystic kidney and liver disease (PKHD1) has been identified on the short arm of chromosome 6. This results in a defect in the fibrocystin protein that exists in bile duct cells, liver, pancreas, and renal tubular cells (4). Its role is in the proper development of the liver and kidneys through controlled cell proliferation (5, 6). The disease is often asymptomatic, and when there are symptoms, they are most often presented with episodes of acute cholangitis, jaundice, fever, high body temperature and abdominal pain below the right costal arch. An increase in bile acids in blood causes itchy skin. These symptoms disappear with aging and the development of liver cirrhosis and portal hypertension. Caroli's disease increases the risk of cholangiocellular liver cancer (4).

The Caroli's disease is diagnosed by taking anamnesis, through clinical examination and non-invasive and invasive diagnostic procedures. Laboratory examination mainly shows elevated levels of leukocytes, alkaline phosphatase and direct (conjugated) bilirubin (7). Abdominal ultrasound is the first diagnostic method that determines sacral enlargements of the intrahepatic bile ducts. Final diagnostics include computed tomography of the liver and biliary tract, invasive methods such as endoscopic retrograde

cholangiopancreatography (ERCP) and magnetic retrograde cholangiopancreatography (MRCP), which confirm the diagnosis of the disease. Due to the connection between cystic changes and liver fibrosis, it is possible to perform a liver biopsy (8, 9).

Case report

We present the case of a 67-year-old male patient who first contacted a gastroenterologist 5 years ago due to pain in the upper half of the abdomen, nausea, vomiting of yellowish contents and fever up to 37.5ºC. The patient felt exhausted and noticed a yellow discoloration of the skin and scleras, and lighter stool color. He denied losing weight, but his appetite was weakened. He also drank alcohol until a couple of years ago. The gastroenterologist indicated hospital treatment when the further diagnosis was made. Laboratory results of the patient at the start of hospitalisation showed elevated values of parameters: CRP 337.9 mg/L, creatinine 801.6 µmol/L, gGT 200 U/L, LDH 254 U/L, ALP 425 U/L, T.Bil 95, 8 µmol/L, D.Bil 64.2 µmol/L. Abdominal ultrasound determined enlarged liver with rounded edges, inhomogeneous parenchyma with numerous transonic changes up to 14 mm in diameter predominantly in the left lobe of the liver, while intrahepatic bile ducts were dilated with the presence of aerobilia. The spleen was enlarged up to 144 mm in interpolar diameter (Figure 1). This raised suspicion of Caroli's disease. In order to confirm the diagnosis, the computed tomography of the abdomen was performed and it verified the enlargement of the right lobe of the liver up to 179 mm, as well as dilated intrahepatic bile ducts filled with multiple calculi up to 1 cm in diameter, predominantly in the right liver lobe - "pearl" sign. The ductus choledochus was dilated in its proximal segment up to 11.5 mm and filled with air, while the diameter of the Wirsung duct was 4.5 mm (Figure 2). This confirmed the Caroli's disease. On several occasions, the patient was examined by a surgeon who did not indicate surgical treatment and due to altered values of acid-base status (pH 7.309, HCO_3 11.3 mmol/L, BE -14.9 mmol/L, Lac 2.15 mmol/L), the patient was treated by a nephrologist. In the next couple of years, the patient regularly visited a gastroenterologist, where he was regularly examined due to similar problems, and on a couple of occasions he was hospitalized again. Proximal endoscopy was performed in order to monitor the disease and it showed polycolor gastric mucosa, mosaic appearance, sometimes with submucosal hemorrhages, without macroscopic changes, while the finding on the esophagus was normal, without dilated esophageal veins (Figure 3). This indicated the possible occurrence of portal hypertension, which is common in patients with Caroli's disease. Physical examination revealed no signs of manifest cirrhosis of the liver. Control computed tomography of the abdomen did not show a significantly different finding on the liver and bile ducts, except for a small amount of free fluid in the abdomen and bilateral effusion in the lungs. The patient was prescribed antibiotic, gastroprotective and hepatoprotective therapy to improve liver function. After a detailed diagnosis, the patient was regularly visiting the clinic for check-ups, but since he stopped coming in 2021, the clinic has not kept a record of him .

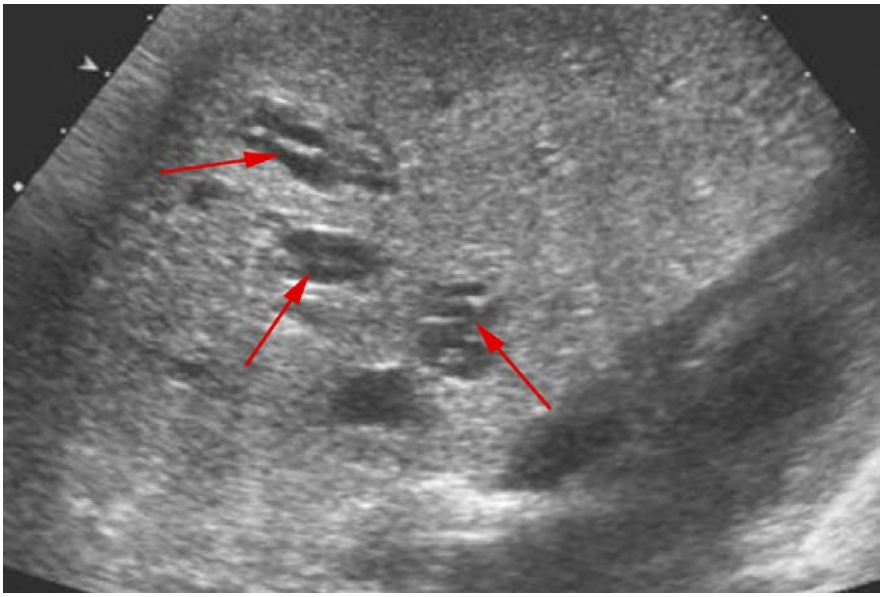

Figure 1. Dilated intrahepatic bile ducts with calculi and aerobilia

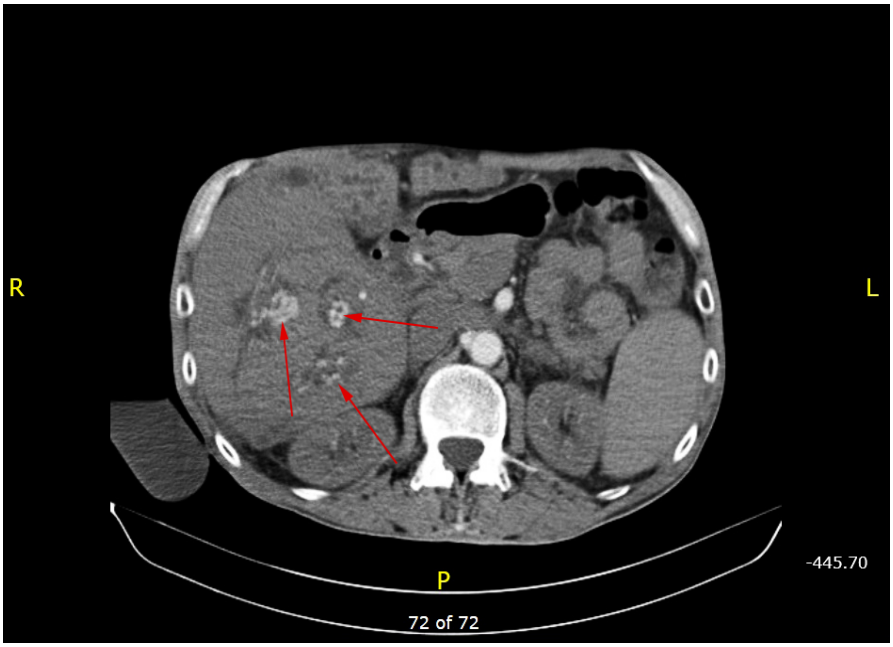

Figure 2. Computed tomography of the abdomen - dilated intrahepatic bile ducts filled with multiple calculi - a sign of "pearls"

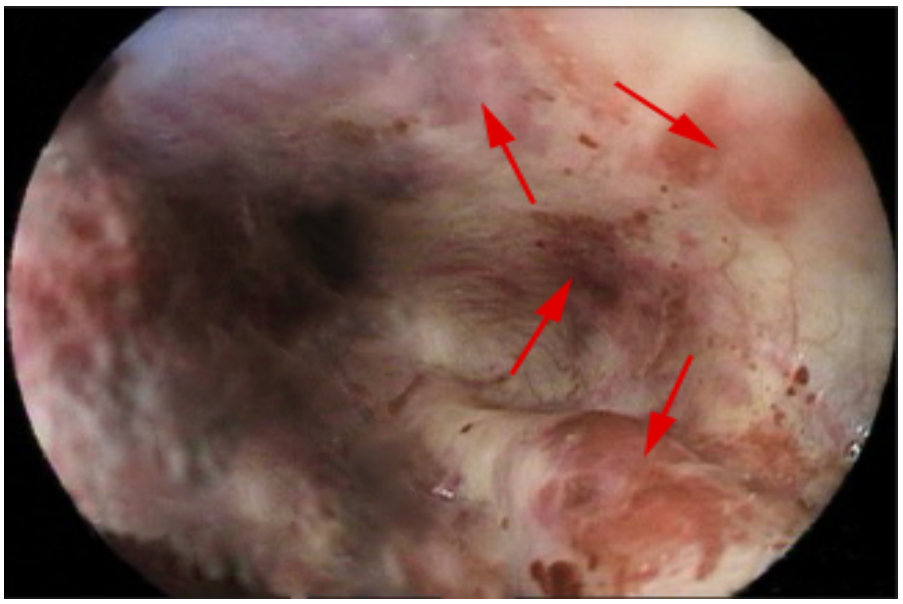

Figure 3. Proximal endoscopy - Polychlorine, mosaic appearance of gastric mucosa with numerous submucosal hemorrhages

Discussion

Caroli's disease is a rare disease of the intrahepatic bile ducts which exact cause is still poorly known (10). It was first described in 1906 by Vachell and Stephens, then in 1958, Caroli J and co-workers gave the disease its final name and defined it as a congenital, cystic liver disorder with dilated intrahepatic bile ducts. Tonadi and co-workers classify Caroli's disease as the fifth type of congenital cysts of the bile ducts (2, 11). These disorders of embryogenesis are often associated with hepatobiliary and renal malformations (11). The most common mutated gene is PKHD1 (gene for polycystic kidney and liver disease) on chromosome 6p12, which participates in the cell differentiation of the liver, kidneys, lungs and pancreas (12). This results in a defect in fibrocystin, which is a part of the cilia of cholangiocytes and represents an important receptor through which chemical, mechanical and

osmotic stimuli trigger intracellular mechanisms that change the composition of bile (13). It also plays an important role in liver and bile proliferation, which explains cystic defects in Caroli's disease (4, 13). Parada and co-workers also found an unbalanced translocation between chromosomes 3 and 8 (loss of 3p and an increase in 8q) in the liver tissue of those patients. It has been suggested that this could be the cause of cholangiocarcinoma development after many years of Caroli's disease (14). In a number of cases, autosomal dominant inheritance has been described together with polycystic kidney disease (15). The disease is usually diagnosed in childhood or adolescence, but also in elderly patients (16). Patients may be asymptomatic for a long time or may occasionally have milder symptoms of the disease (17). The most common symptoms are abdominal pain, fever and repeated attacks of cholangitis, and with disease progression, signs of portal hypertension such as haematemesis and melena may occur (18). The fact that there is no exact age when the disease develops was confirmed by a study by Bisvas and co-workers who showed that the most common period of illness is from 2 to 16 years of age, with an average age of 10 years. These younger patients developed a serious clinical picture in the form of fever and jaundice, while ultrasound examination revealed numerous bile calculi in the dilated bile ducts, which is the case in our elderly patients (19). Bile stasis and chronic cholangitis are predispositions for the development of biliary tract dysplasia and cholangiocarcinoma, which occurs in 7% (according to some studies from 5 to 33%) of patients with Caroli's disease and is the most severe complication of this disease (20, 21). Cases of hepatocellular and gallbladder cancer have been reported, although this is significantly less common (20, 22). The risk of developing cholangiocellular carcinoma in Caroli's disease and Caroli's syndrome is about 100 times higher compared to healthy individuals. This development of cancer was first described in the literature in 1968, but the etiology is still not completely clear (23). However, there are a couple of theories: long-term bile stasis followed by bacterial infection, irritation of the calculi and release of carcinogenic substances may participate in the development of cholangiocarcinoma. It is assumed that older age and long duration of the disease contribute to its development (23, 4). A study by Ghadir Mohammad and co-workers shows cases of two elderly patients in England who developed cirrhosis of the liver after numerous years, and one of these two patients also developed hepatocellular carcinoma. Although this review reveals an elderly patient with initial signs of portal hypertension, numerous other studies show that these complications are also possible in significantly younger patients (25).

Beside the clinical examination, the diagnosis is made by ultrasound examination, which shows accuracy in only 27.3% of cases, so it is much more often recommended to use computed tomography of the abdomen to verify changes in the bile ducts with an accuracy of 71.4%. This method verifies the “central point” sign which is representing the fibrovascular bundles containing the portal vein and artery around the dilated bile ducts (26). Today, the traditional method of diagnosis by ERPC (endoscopic retrograde cholangiopancreatography) is increasingly being replaced by MRCP (magnetic resonance cholangiopancreatography) and is becoming the method of choice for diagnosing Caroli's disease (30). Its non-invasive nature is detecting malignancy make it the most effective method. The MRCP method has a sensitivity and specificity of 97 to 99% (26, 27). This method is effective in the differentiation of Caroli's disease from similar conditions such as benign biliary cysts, choledochus cysts and polycystic kidney disease (26).

Treatment of Caroli's disease includes the use of antibiotics (in the case of cholangitis) and ursodeoxycholic acid in the case of severe lithiasis. Surgical resection is useful in patients with segmental or monolobar disease in the absence of recurrent cholangitis, advanced fibrosis, or cirrhosis (28). The most commonly used surgical methods are endoscopic sphincterotomy, cholecystectomy and resection of the affected liver lobe - lobectomy, segmentectomy (29). In the case of advanced disease, development of fibrosis, liver cirrhosis and portal hypertension, the most effective method of treatment is liver transplantation (30).

Conclusion

Caroli's disease is a rare disease of the biliary tract. The exact incidence of this disease is not known because a large number of cases are asymptomatic, so the disease is rarely diagnosed. This disease can occur throughout life, but most often in adolescence and in elderly patients. In order to discover the disease in the early stage it is necessary to conduct right diagnosis and to hospitalize the patient with the appearance of the first symptoms of the disease. Although there is no reliable therapy for liver fibrosis and cirrhosis, the use of hepatoprotective drugs and changes in lifestyle habits could at least slow down the further progression of the disease. In case of complications of developed portal hypertension, rapid endoscopic interventions would increase survival in later stages. Regular monitoring of these patients could reduce the risk of complications and increase the survival of this rare disease.

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Prikaz bolesnika

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KAROLIJEVA BOLEST – BOLEST O KOJOJ SE MALO RAZMIŠLJA: PRIKAZ BOLESNIKA

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Karolijeva bolest je retko oboljenje, koje karakteriše dilatacija velikih intrahepatičnih žučnih kanala. Javlja se u retkoj izolovanoj formi, koju odlikuju ponovljene epizode holangitisa i periportalna fibroza (tip I). Znatno češća varijanta povezana je sa kongenitalnom fibrozom jetre (tip II). Iako se bolest može javiti tokom čitavog života, najčešće se javlja pre tridesete godine. Incidencija i prevalencija ove bolesti nisu poznate, ali je procena da se javlja jednom u 10.000 do 20.000 slučajeva. Glavni simptomi su povišena telesna temperatura, bol ispod desnog rebarnog luka, napadi žutice, svrab po koži, mučnina i povraćanje. Dijagnostika se vrši kliničkim pregledom, ultrazvučnim pregledom, kompjuterizovanom tomografijom abdomena, a u cilju definitivne dijagnostike primenjuju se endoskopska retrogradna holangiopankreatografija i biopsija jetre. U slučaju razvoja ciroze jetre i portne hipertenzije radi se proksimalna endoskopija. Prikazujemo klinički slučaj čoveka starijeg životnog doba, koji nekoliko godina unazad boluje od Karolijeve bolesti. Prikazani su dijagnostički izazovi i primenjena terapija. *Acta Medica Medianae 2023;62(2):77-82.*

Ključne reči: *Karolijeva bolest, holangitis, fibroza jetre, ciroza jetre*

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Case report

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CENTRAL DURAL TENTING SUTURES IN A PATIENT WITH TRAUMATIC ACUTE EPIDURAL HEMATOMA: A CASE REPORT

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It is of paramount significance to diagnose epidural hematoma (EDH) as soon as possible and to evacuate EDH if necessary. Our aim was to show the importance of placing prophylactic central dural tenting sutures (DTS) for ensuring adequate hemostasis. We present a case of a 62-year-old male patient, who was diagnosed with an acute traumatic EDH and cerebral hemorrhagic contusion. The postoperative course was complicated by deterioration of the patient's consciousness and worsening of the left-sided hemiparesis, therefore we performed a head CT scan, which showed the formation of the new EDH at the operating site. Moreover, we reoperated the patient with the placement of multiple central DTS, while the control head CT scan showed a complete evacuation of the EDH. Consequently, the patient made a good recovery at discharge, with a remaining discrete left-sided hemiparesis. Prophylactic central DTS are important for maintaining an adequate hemostasis while operating on patients with EDH. *Acta Medica Medianae 2023;62(2): 83-87.*

Key words: *epidural hematoma, dural tenting suture*

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Introduction

Epidural hematoma (EDH) is an extra-axial collection of blood in the space between the outer sheet of the dura mater and the inner wall of the skull (1). It represents a life-threatening and urgent condition that requires rapid and adequate treatment. EDH occurs in 2.7–4% of cases of traumatic brain injury (TBI) and 9% of cases of severe TBI, with an estimated mortality of approximately 10% (1, 2). EDH can occur as a result of injury to the middle meningeal artery (90%), middle meningeal vein, diploic veins or venous sinuses and can be clinically manifested in different ways, ranging from comatose to fully conscious state (3). The decision for operative treatment is usually based on the evaluation of various clinical, systemic and radiological findings. Neurological examination includes assessment of Glasgow Coma Scale (GCS), pupillary findings and motor function (4). Furthermore, there is no clear consensus or guidelines on what occasion should central DTS be applied, while certain procedures in this operative technique are non-standardized.

Case report

The authors present a 62-year-old male patient, who underwent surgery for an EDH located below the right parietal bone, with the largest width up to 15 mm, as well as a cerebral hemorrhagic contusion (CHC) measuring 37x25 mm in the parietal lobe.

Moreover, he allegedly sustained the previously described injuries after a syncope, when he fell headfirst on the floor, injuring the right parietal side of his head. During the neurosurgical examination in the emergency room, on admission, the patient had a progressive alteration of the state of consciousness reaching soporous state (GCS score of 9), without gross lateralization of neurological deficits on the extremities or cranial nerves. Henceforth, the grand mal seizures were observed, although he did not suffer from any preexisting seizure

disorder. The diagnosis was initially confirmed by a head computerized tomography (CT), where, in addition to the above-mentioned EDH and CHC, two parallel and linear fractures of the right parieto-temporal bone and subarachnoid hemorrhage were demonstrated (Figure 1). After an adequate preoperative examination, the patient underwent an emergency surgery. During the operation, the patient was placed in the supine position under general anesthesia. The head was elevated above the level of the heart to promote venous outflow and reduce intracranial pressure. The patient's head was rotated to the left side (0°–15° from the horizontal plane). The roll was placed under the right shoulder to facilitate the head turning. Moreover, the head was supported with a Mayfield standard cranial stabilization system. The ipsilateral area of interest was shaved, adequately disinfected and draped. A trauma-flap (reversed question mark incision) approach was commenced, ending behind the hairline area. The temporalis musculocutaneous flap was elevated and craniotomy was performed with the five points of trepanation. The EDH was evacuated subsequently. The bleeding from the diploic veins and middle meningeal artery was identified and then coagulated and stopped by using hemostatic materials. The durotomy was performed in a semicircular fashion and the CHC was evacuated by using a gentle suction and irrigation. Hemostasis was managed with the use of regenerated oxidised cellulose (Surgicel®) and bipolar electrocautery. The subdural drainage was placed. The dura was watertight closed and DTS were placed on the surrounding post-craniotomy margins to prevent a re-accumulation of the hematoma. An epidural drain was placed while the bony lid was returned and fixated with non-dissolvable sutures.

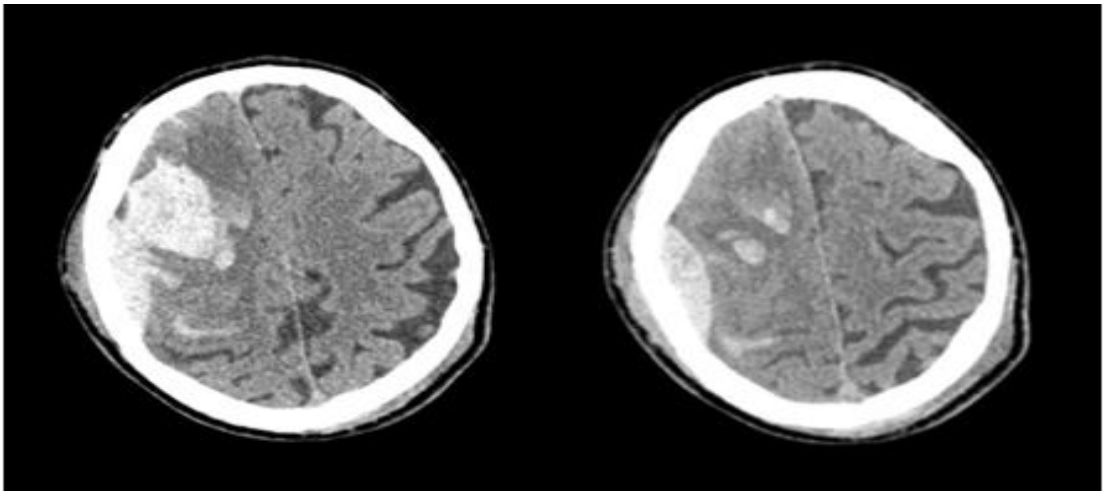

Figure 1. CT tomograms demonstrate a right parietal EDH with the greatest width up to 15 mm, CHC with dimensions of 37x25 mm in the right frontal and parietal lobe, as well as diffuse subarachnoid hemorrhage.

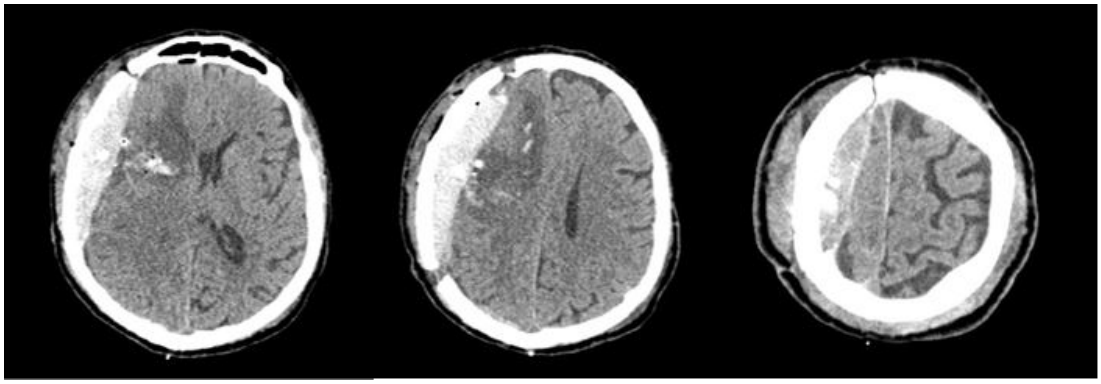

Figure 2. CT tomograms demonstrate the presence of expansive acute EDH with the largest width of 22 mm. Encephalomalacia in the region of the evacuated CHC and ischemic zone in the vascular territory of the MCA on the right are also evident.

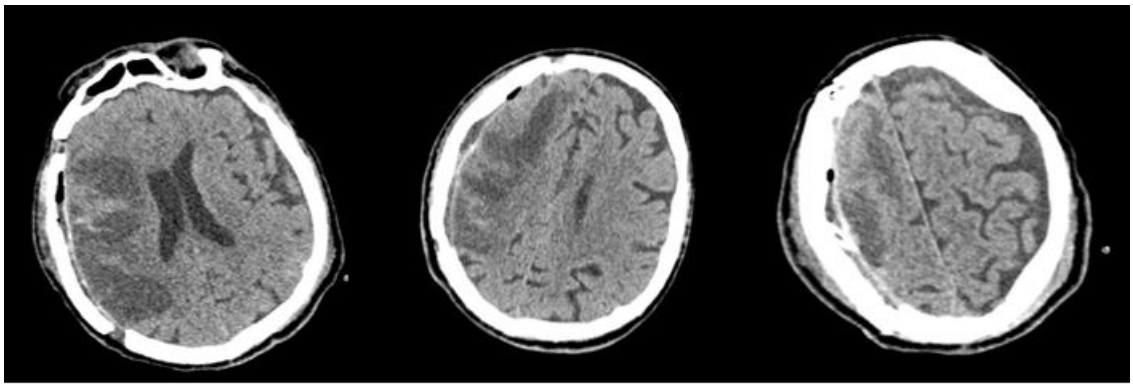

Figure 3. CT tomograms demonstrate a resolved EDH and an ischemic zone in the vascular territory of the right MCA.

Postoperatively, due to the development of flaccid left-sided hemiplegia, a control head CT was performed, which recorded the size progression of EDH in the region of the previous operation, with a maximum width of up to 22 mm, as well as ischemia in the vascular zone of the right middle cerebral artery (MCA) (Figure 2).

Upon receiving the results of the control head CT, we performed an urgent reoperation.

Moreover, the bleeding was originating from the Pachyoni's corpuscles, which was stopped with the use of bipolar electrocautery, while 5 central DTS were additionally placed.

The control head CT scan showed an evacuation of the EDH with a persistence of the ischemic zone in the area of the right MCA (Figure 3).

Consequently, the patient made a good recovery at discharge, with a remaining moderate spastic left-sided hemiparesis. Follow-up examinations were performed after 3 and 6 months, as well as after 1 year, while the patient reached a satisfying recovery. The patient was walking independently without the use of a cane or walker, and the gross motor activities of the left half of the body reached grade 4 on the Manual muscle test scale after physical and rehabilitation treatment.

Discussion

Traumatic delayed EDH accounts for 5.6% to 13.3% of all cases and represents EDH that is absent or of insignificant dimensions on initial brain CT and MRI, while subsequent imaging shows significant EDH (5). In rare cases, EDH may be overlooked because of its small size on initial head CT or because the density of the acute hemorrhage may be similar to that of the skull (6). On the contrary, our patient had a recurrence of EDH because epidural bleeding was shown during the initial radiological imaging and it had substantial dimensions.

In the early years of neurosurgical development, hemostasis was the dominant technical problem during surgical evacuation of EDH. Horsley's wax and Cushing's electrocautery were major contributions to neurosurgery, as well as Dandy's DTS. Moreover, DTS were used to attach the dura tightly to cranial bone and to prevent to some extent the recurrence of EDH, by reducing the volume of the potential epidural space and collapsing the dural blood vessels (7, 8).

Despite the large number of published research in the scientific literature on the application of DTS, there are still no official guidelines on indications, application time during the surgery and placement techniques of central DTS.

Vadanmbi et al. analyzed the necessity for DTS placement in 785 patients who underwent craniotomy and craniectomy, while obtaining the result that DTS do not statistically significantly affect the reduction of extradural postoperative bleeding requiring surgical reoperation compared to the group of patients in whom DTS was not placed (9). We believe that the limitations of their research were that they did not include the placement of central DTS, patient comorbidities, coagulation factor screening, the severity of the underlying disease that required craniotomy and craniectomy, as well as that there were huge differences in the age of the patients.

The use of DTS often leads to the appearance of certain complications, such as acute subdural hematoma, subdural hygroma, cerebrospinal fluid leakage, cortical tissue damage (1).

Placement of central DTS immediately before closing the dura or using a microscope significantly reduces the risk of complications related to DTS (1, 10). Nishiyama et al. reported a rare complication of pial arteriovenous fistula formation after DTS placement (11). A possible explanation for the occurrence of this complication could be that the authors of this study probably stimulated abnormal angiogenesis by cytokine releasement and vascular growth factors through mechanical injury of the MCA or Sylvian vein. Given that we had a clear visualization of the

cerebral cortex and bridging veins, as well as CHC after opening the dura, we believe that there was no need to use the operating microscope. The use of a microscope certainly increases the chance of successfully treating and visualizing traumatic brain injuries, while on the other hand it prolongs the duration of the operation, and in our patient, the time required for hematoma evacuation was of crucial importance.

Bearing in mind the hemostatic role and the complications that DTS can cause, possible alternatives for central DTS should be considered in the future. Additionally, a new technique of implanting self-drilling screws, after which dural sutures can be tied around the screw heads, thus serving as anchors has been proposed. This technique has shown satisfactory results in supraorbital keyhole craniotomy, sphenoid ridge keyhole craniotomy, pterional and subtemporal approaches (12). On the other hand, some authors suggest benefits of using a procedure called bone hole threader, which goes through the hole of the bony lid, with a wire tip that is elastic and thus prevents injury to the surrounding tissue. The potential benefits of this procedure could be that it reduces the average central DTS placement time by 24 seconds and allows the operator to place the central DTS without the assistance (13).

Specifically, in this case presentation, by opening the dura, a better visualization of the cortex and bridging veins was achieved, which minimized the risk of potential damage during central DTS placement. However, we believe that further research regarding this topic and operative technique is necessary, in order to establish a consensus and guidelines for the use of central DTS.

Conclusion

DTS placement has a hemostatic role and has been used in neurosurgery for decades. Central dural suspensions are of great importance after durotomy in cases of EDH, subdural and intracranial hemorrhages, in prevention of possible additional detachment of the dura and accompanying complications. However, there are very few studies on this issue, so we believe that additional research on the use of DTS, operative techniques and potential alternatives are necessary.

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Prikaz bolesnika

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ZNAČAJ CENTRALNIH DURALNIH SUSPENZIJA KOD BOLESNIKA SA AKUTNIM TRAUMATSKIM EPIDURALNIM HEMATOMOM: PRIKAZ SLUČAJA

Aleksandar Marković[1], Jovan Ilić[1], Ivan Stefanović[1,2], Vesna Nikolov[1,2], Miša Radisavljević[1,2], Jelena Kovačević[1], Nikola Stojanović[1], Marija Đorđević[2]

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Traumatski epiduralni hematom (EDH) je potrebno dijagnostikovati što pre i, ukoliko je potrebno, razmotriti njegovo operativno uklanjanje. Cilj našeg rada bio je da ukažemo na važnost postavljanja profilaktičkih centralnih duralnih suspenzija (DS) u cilju obezbeđivanja adekvatne hemostaze. Predstavljamo slučaj šezdesetdvogodišnjeg bolesnika kod koga je postavljena dijagnoza akutnog traumatskog EDH i cerebralne hemoragične kontuzije (CHK). Bolesnik je hitno operisan – tada je učinjena evakuacija EDH i CHK, uz postavljanje perifernih DS. Postoperativni tok zakomplikovao se pogoršanjem stanja svesti bolesnika do nivoa sopora i pogoršanjem levostrane hemipareze, te je načinjen kontrolni CT mozga, koji je pokazao ponovni EDH na mestu prethodne operacije. Sledstveno, bolesnik je reoperisan uz postavljanje višestrukih centralnih DS, a kontrolnim postoperativnim CT-om mozga evidentirana je potpuna evakuacija EDH. Posledično, bolesnik je na otpustu dostigao zadovoljavajući oporavak, sa rezidualnom, diskretnom levostranom spastičnom hemiparezom. Profilaktičke centralne DS važne su za postizanje adekvatne hemostaze tokom operacije pacijenata sa EDH. *Acta Medica Medianae 2023;62(2):83-87.*

Ključne reči: *epiduralni hematom, duralna suspenzija*

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Case report

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COMBINED STABILIZATION OF THE PELVIC RING DISRUPTION INCLUDING TECHNIQUE OF SACRAL BARS: A CASE REPORT

Igor Merganoski[1,2], Simon Trpeski[1,2], Oliver Arsovski[1,2], Igor Kafrandžiev[1,2], Marko Spasov[1,2]

The aim of this case presentation is to demonstrate that the technique of sacral bars, combined with anterior fixation with a plate, is an effective method of posterior pelvic stabilization in cases of severe pelvic ring disruption.

We are presenting a case of a young individual who sustained severe pelvic ring disruption (Type A III according to the Tile classification) with sacral fracture following compression injury of the lower torso. Initially, explorative laparotomy for splenic injury was performed, while surgery for pelvic stabilization was postponed for 5 days. The surgery for pelvic stabilization included fixation of the symphysis with a plate followed by posterior fixation with two sacral bars. No postoperative complications were noted. The patient was followed for a year post injury, and he made full recovery returning to complete preinjury level of activity

Posterior stabilization with sacral bars in pelvic ring disruptions combined with anterior plate of the symphysis is safe and effective method for the treatment of this type of injury. *Acta Medica Medianae 2023;62(2): 88-93.*

Key words: *pelvis, sacrum, disruption, sacral bars, fracture*

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Introduction

Surgical stabilization of posterior pelvic disruption which is frequently combined with sacral fractures is still a challenge for both the spine and trauma surgeons.

Sacral bars, inserted following open reduction, represent a recognized method for fixation of sacroiliac joint disruption and/or sacral fractures with or without anterior pelvic ring disruption.

Sacral fractures are commonly classified with regard to the location of the sacral fracture. Type I fractures involve the sacral ala, type II fractures involve the sacral foramina, and type III fractures involve the central portion of sacrum (1). Roy-Camille has further sub-classified central sacral fractures. Operative stabilization of sacral fractures is indicated in the fractures that are displaced, that result in pelvic ring instability and the sacral fractures with foraminal debris causing a neurologic deficit (2).

Approximately 10% of all patients who sustain pelvic fractures present with neurological deficit. Most neurologic injuries involve the L5 and S1 nerve roots of the lumbosacral (LS) plexus; however, a significant number of patients also experience sexual dysfunction secondary to nerve injury of the lower sacral nerves (3) (4).

We present a case of a 16 years old healthy male patient with bilateral sacrum fracture combined with anterior pelvic ring disruption (disruption of symphysis pubis and fracture of left pubic rami). The patient underwent open reduction and internal fixation with two sacral bars.

Case presentation

A 16 years old healthy male patient was brought by an ambulance to our Emergency department after being buried by a large quantity of mud predominantly in the region of abdomen and pelvis while working by a river, which caused compression-type injury to his lower trunk. On admission, the patient was alert and hemodynamically stable. Physical examination demonstrated many abrasions and tenderness in the upper abdomen, as well as abrasions, tenderness and instability in the region of the pelvis and the lower back bilaterally. Pelvic examination by compression demonstrated

instability in both planes, while at this point neurological status demonstrated no deficit. The extremities were without deformities and abnormalities.

Anteroposterior X-ray of the pelvis revealed bilateral sacrum fracture combined with anterior pelvic ring disruption (disruption of the symphysis pubis and fracture of the left pubic rami) (Figure 1). Ultrasonography illustrated free fluid in the pelvis, also some fluid around the spleen with direct ultrasonography signs of spleen injury. Contrast CT revealed a stable retroperitoneal hematoma, laceration of the spleen and a displaced bilateral fracture of the sacrum (Denis grade II-III), disruption of the symphysis pubis (APC III) and fractures of the left pubic rami. The pelvic fracture was classified as A III according to Tile's classification (Figure 2).

Initially, the patient was admitted in the Intensive care unit, following which, emergency laparotomy was conducted. The surgery for pelvic injury was performed five days following injury.

The surgical plan included anterior pelvic fracture reduction and plate fixation followed by posterior pelvic fixation using sacral bars (Figure 3).

We used the anterior Pfannenstiel approach with anatomical reduction followed by symphyseal plate fixation. After closure of the anterior wound in layers, the patient was brought to prone position. We made two curvilinear incisions, at the ilio-sacral level. After getting a clear view, we inserted two titanium bars that were pointed away from the foramina and introduced in the dense sacral bone. Because of the threatening hazard of damage to the neighboring neurological structure, only partial reduction was performed to fix the sacral fracture (partial but quite enough to keep the pelvis stable and to give good fracture union) (Figures 4 and 5). Our total operative time was 80 minutes including both anterior and posterior fixation.

Postoperative X-rays demonstrated satisfactory anatomical reduction (Figure 6). We had no postoperative complications and the patient was discharged from our hospital on the 7th postoperative day. The patient underwent regular postoperative rehabilitation protocol, regular follow ups were conducted one month, three months, six months and a year post surgery. He made a full recovery and returned to his regular activities and work six months post injury.

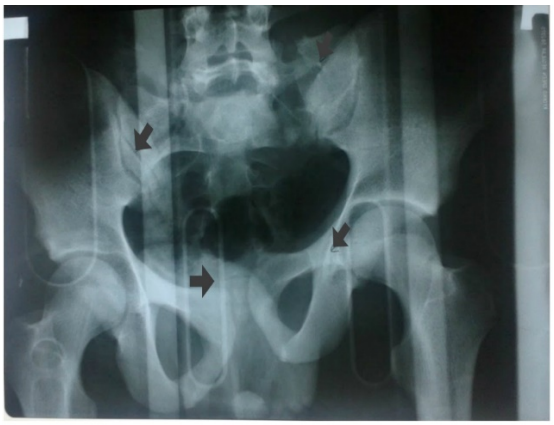

Figure 1. AP X-ray on admission, bilateral sacrum fracture combined with anterior pelvic ring disruption (disruption of the symphysis pubis and fracture of the left pubic rami)

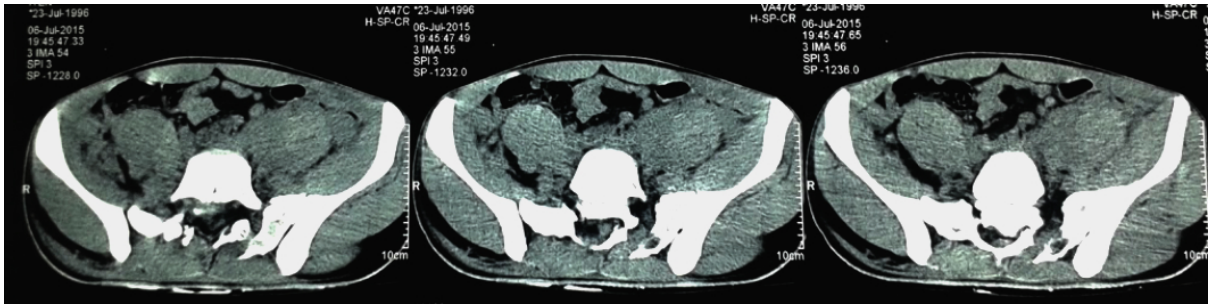

Figure 2. Contrast CT on admission, displaced fracture of the bilateral sacrum (Denis grade II-III)

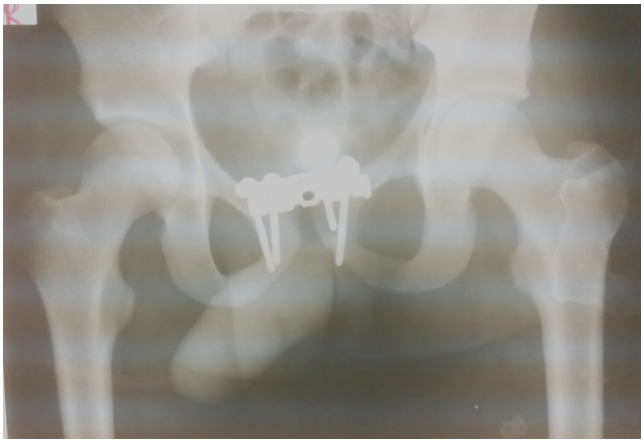

Figure 3. AP X-ray after fixation of the symphysis pubis

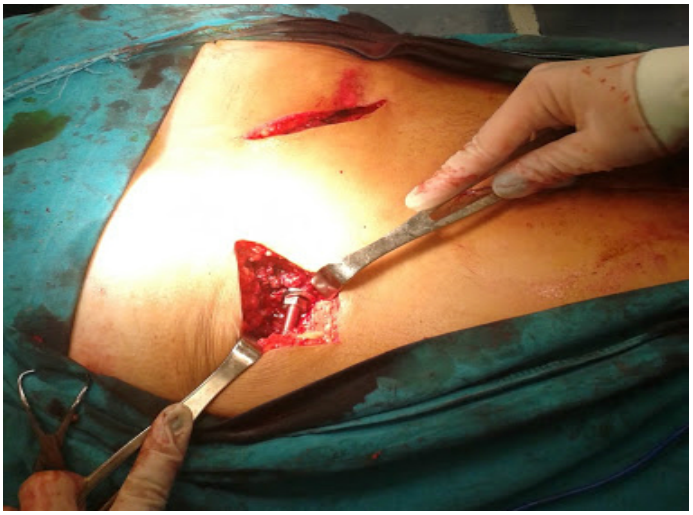

Figure 4. Open fixation on posterior pelvic ring with bars and screws introduced into sacral bone

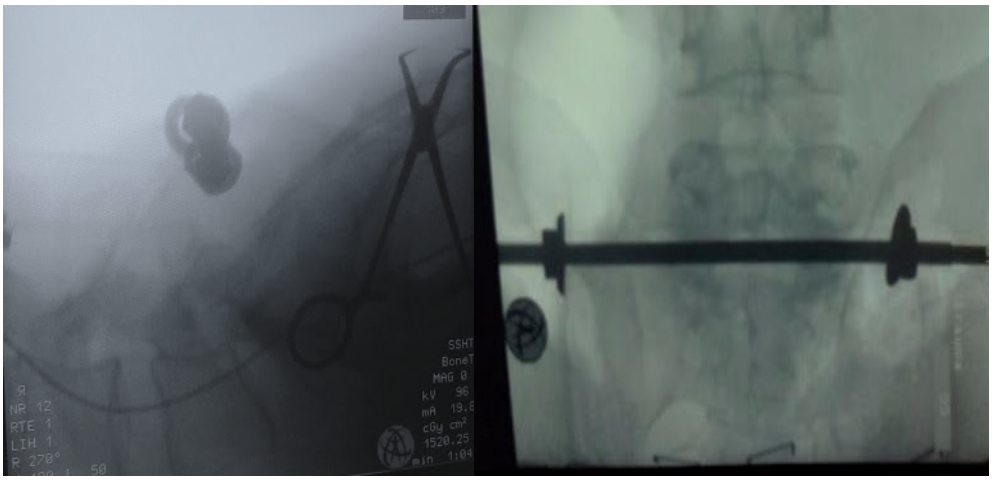

Figure 5. Profile and AP intraoperative X-ray of the pelvis after inserting the first bar and screw

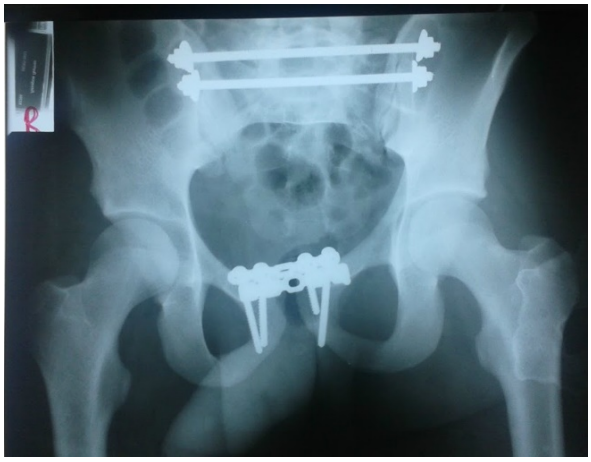

Figure 6. Postoperative AP X-ray showing excellent anatomical reduction of anterior and posterior pelvic ring

Discussion

Fixation of the sacrum with the technique of sacral bars has not been widely used in our hospital, nor in our country. This technique of fixation has its own advantages and disadvantages. The main advantage is stable fixation on the posterior component of the pelvic with the implant placed behind the distal lumbar spine and sacrum, thus avoiding potential injury to nerve roots and the central sacral canal that lie anteriorly (5).

The disadvantages of this procedure include: mal-reduction, breakage of bars due to limited biomechanical strength, LLD, instability, compressive neuropathy of sacral roots or cauda equina, injury to vascular or intestinal structures, lower back pain and infection (6, 7, 8, 9, 10).

Preoperative radiological evaluation must include AP and lateral X-rays, inlet and outlet views and CT, with/without 3D. Some surgeons already use intra-operative CT or navigation for percutaneous ilio-sacral bar placement (8, 11).

In order to reduce the damage of sacral roots and nerves, some surgeons use intra-operative monitoring with stimulus-evoked EMG, especially when introducing ilio-sacral screws (12).

Conclusion

Sacral bar osteosynthesis is a promising method for stabilization of fractures of the pelvic ring. Only with this method, a high interfragmentary compression is achieved, independent of the quality of the spongious bone of the sacral body (13).

The importance of this case presentation is in encouraging the surgeons in Macedonia and in the region, especially the ones who work in level one trauma care setting, or in bigger hospital that can deal with polytraumatized patients, to perform this kind of technique more often, but only with the right indications for this procedure. In conclusion, the use of sacral bars is a safe and effective method for posterior pelvic fixation and should be used with right indications.

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KOMBINOVANA STABILIZACIJA DISRUPCIJE KARLIČNOG PRSTENA UKLJUČUJUĆI TEHNIKU SAKRALNIH ŠIPKI – PRIKAZ SLUČAJA

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Cilj ovog prikaza slučaja jeste ukazivanje na to da je tehnika sakralnih šipki efikasna metoda posteriorne stabilizacije karlice u slučajevima ozbiljnog poremećaja karličnog prstena, kada se kombinuje sa prednjom fiksacionom pločom.

Predstavljamo slučaj mlade osobe koja je pretrpela teški poremećaj karličnog prstena (Tip A III prema Tile klasifikaciji), sa sakralnim prelomom, nakon kompresione povrede donjeg dela trupa. U početku je urađena eksplorativna laparotomija, zbog povrede slezine, dok je operacija stabilizacije karlice odložena za pet dana. Operacija stabilizacije karlice podrazumevala je fiksaciju simfize pločom, a potom posteriornu fiksaciju dvema sakralnim šipkama. Nisu zabeležene postoperativne komplikacije. Bolesnik je praćen godinu dana i potpuno se oporavio, vraćajući se na potpuni nivo aktivnosti, kao pre povrede.

Posteriorna stabilizacija sakralnim šipkama kod disrupcije karličnog prstena i kombinacija sa prednjom pločom simfize sigurna je i efikasna metoda za lečenje ove vrste povrede. *Acta Medica Medianae 2023;62(2):88-93.*

Ključne reči: *karlica, sakrum, disrupcija, sakralne šipke, prelom*

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JEDINSTVENI KRITERIJUMI ZA OBJAVLJIVANJE NAUČNIH RADOVA U BIOMEDICINSKIM ČASOPISIMA

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

Kriterijumi za citiranje i navođenje referenci

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

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

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

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

Kriterijumi za pisanje referenci korišćenih u radu

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

Članci u časopisima

1. Standardni članak u časopisu

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

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

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

2. Organizacija kao autor

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

3. Članak bez poznatih autora

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

4. Volumen sa suplementom

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

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

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11. Monografija

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12. Autori kao urednici

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

13. Organizacija kao autor i izdavač

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

14. Poglavlje u knjizi

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

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

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

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Službeni izveštaji (Issued by funding / sponsoring agency):

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

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

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

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U tekstu naznačiti mesta priloga i obeležiti ih onako kako su obeleženi u prilogu.

Literatura se daje u posebnom poglavlju, pri čemu se navodi onim redosledom kojim se citati pojavljuju u tekstu. Broj literaturne reference se u tekstu označava arapskim brojem u zagradi. Za navođenje literature koristiti pravila Vankuverske konvencije. Strane se numerišu arapskim brojevima u donjem desnom uglu

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

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Ukoliko je tabela ili ilustracija već negde objavljena, citirati izvor i priložiti pismeno odo-brenje, ukoliko se radi o zaštićenom materijalu. Ukoliko je na fotografiji prikazan bolesnik tako da se može prepoznati, potrebno je njegovo pismeno odobrenje, u suprotnom, delovi fotografije se moraju izbrisati da bolesnik ne može biti identifikovan.

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