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5. Belenkaya RM. Structural variants of the brain base arteries. Vopr neirokhir 1974; 5:23–29. (Russian)

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7. Patten MB. Human embryology, 3rd edn. McGraw-Hill: New York, 1968.
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11. Apostolides PJ, Lawton MT, David CA, Spetzler RF. Clinical images: persistent primitive trigeminal artery with and without aneurysm. Barrow Quarterly 1997; 13(4). http://www.thebarrow.org/Education_And_Resources/Barrow_Quarterly/204843
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HYPERLIPIDEMIA IN ACUTE PANCREATITIS: CONCOMITANT DISORDER OR A CAUSE?

Milan Radojković[1*], Miroslav Stojanović[1], Danijela Radojković[2], Ljiljana Jeremić[1], Goran Stanojević[1], Zoran Damnjanović[3], Goran Stevanović[1]

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Abstract. *Acute pancreatitis is a common condition with alcohol and gallstones being the most frequent etiologies. The aim of our study was to determine the prevalence of hyperlipidemia, its etiopathogenetic role and influence on outcomes in patients with acute pancreatitis.The study included 47 patients admitted to our clinic for acute pancreatitis during one year period. On admission patients with hyperlipidemia were compared to those without it, regarding following parameters: body mass index, Glasgow score, organ failure occurrence, local complications occurrence (pancreatic necrosis, pseudocyst, abscess, jaundice, gastric outlet syndrome), intensive care unit stay and death. The results of the study revealed high incidence of hyperlipidemia in 51% of examined acute pancreatitis patients with the prevalence of severe forms in more than half of these patients. Dominant lipid disorder was hypertriglyceridemia, followed by hypercholesterolemia. It was clearly demonstrated that patients with hyperlipidemia, especially hypertriglyceridemia, had more severe acute pancreatitis, higher incidence of complications and poorer outcome compared to normolipemic patients. Hyperlipidemia in patients with acute pancreatitis should be considered and treated by a clinician as a separate serious problem, both when being a cause and a concomitant disorder. Hypolipidemic therapy should be administered both in urgent acute pancreatitis settings and as a long-term treatment aimed to prevent inflammation recurrence by successful persistent serum lipid levels control.*

Key words: *Hyperlipidemia, acute pancreatitis*

Introduction

Acute pancreatitis (AP) represents a serious clinical issue in everyday surgical practice. Alcohol and gallstones are the most frequent etiologies of this potentially severe condition. Other causes, like metabolic, structural and iatrogenic, account for up to 25% of AP cases [1].

Yadav and Pitchumoni [2] remarked that in 1846 Speck [3] was the first who described an association of the hyperlipidemia (HLP) and AP. However, since then etiological correlation of lipid disorders and AP still remains unclear. Hyperlipidemia may be an epiphenomenon to AP, since secondary lipid abnormalities are commonly found in patients with alcohol-induced AP and diabetic, pregnant and obese patients [4]. Nevertheless, primary lipid disorders, especially hypertriglyceridemia (HTG) or chylomicronemia, may independently induce AP and are responsible for up to 7% of cases [1]. Contrary to HTG, hypercholesterolemia does not cause AP [2]. Also, it is still uncertain whether HLP influences the evolution and outcome of AP. In available literature it has been suggested both that the presence of HLP is associated with more severe forms of AP [5] and, alternatively, that it does not intensify the course of inflammatory disease [6]. Cameron et al. reported that association between lipid abnormalities and AP is more than coincidental stating that lipids may play an intermediary role in the pathogenesis of this disease [7].

The aim of our study was to determine the prevalence of HLP, its etiopathogenetic role and influence on outcomes in patients with AP.

Material and Methods

The data were collected prospectively and the study included patients admitted to our clinic for AP during one year period with serum amylase level two times higher than normal range (28–100 U/L) considered confirmatory [8]. Patients previously treated elsewhere for actual AP episode and those with exacerbated chronic pancreatitis and pre-existing organ failure were excluded. On admission complete laboratory investigation was performed and Body mass index (BMI) was calculated (kg/m2). Lipid status assessment was done within 24h of admission and included measurement of triglycerides (TG), cholesterol, low density (LDL) and very low density lipoproteins serum levels. Radiological "imaging" assessment included abdominal ultrasound within 48h of admission and multi-slice computed tomography

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examination performed on the 6th day after disease onset. The aetiology of AP was ascertained from history and collected examination data and disease severity was assessed by the Glasgow criteria and the occurrence of organ dysfunction. Lipid status assessment was repeated on discharge and, if elevated on discharge, serum lipids were measured again one month after discharge. Patients were categorized according to Fredrickson classification of HLP.

Patients with HLP on admission were compared to those without HLP regarding following parameters: BMI, Glasgow score (GS), organ failure occurence (OF), local complications (LC) occurrence (pancreatic necrosis, pseudocyst, abscess, jaundice, gastric outlet syndrome), intensive care unit (ICU) stay and lethal outcome (LO). The data were statistically analyzed using nonparametric Pearson's chi-squared test.

Results

From January 2012 to January 2013 there were 47 patients — 29 (62%) males and 18 (38%) females with average age of 41 years (range, 19–76) admitted for AP. Alcohol was the cause in 25 (53%) and gallstones in 22 (47%) patients. The incidence of potential additional etiological factors was as follows: two patients were taking thiazide diuretics, one estrogen contraceptive and one was on statins therapy, seven were diabetic. Six patients stated that one or both of their parents have had HLD, but were not able to provide precise data about the form of HLP, its duration, intensity and outcome, which (with inability to perform genetic investigation) was not enough to diagnose familial HLP. The incidence and distribution of hyperlipidemic disorders are presented in Table 1; the prevalence of examined parameters is presented in in the Figure 1.

Table 1. The incidence and distribution of hyperlipidemic disorders

Type of hyperlipidemia	No of patients: 24
Hypercholesterolemia	8 (33%)
Moderate	3
Severe	5
Hypertriglyceridemia	16 (67%)
Moderate	7
Severe	9
Transient (normalized at discharge)	11 (46%)
Persistent (one month after discharge)	8 (33%)
Lethal outcome	5 (21%)

Out of 10 patients with GS>3 seven had HLP, out of 10 patients with organ failure six had HLP, out of 17 patients treated in ICU 12 had HLP, out of 14 patients with local complications 10 had HLP and out of six patients that died five had HLP (Fig. 2).

The correlation between the duration of HLP (transient or persistent) and severity, course and outcome of AP is presented in the Figure 3. The analysis of correlation between the severity of HLP and severity, course and outcome of AP revealed similar pattern (Table 2).

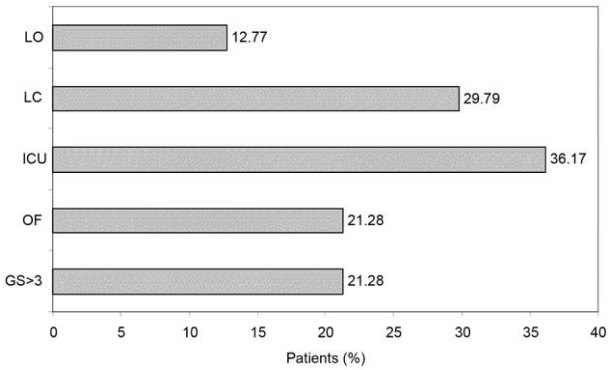

Fig. 1. The prevalence of examined parameters.
LO, lethal outcome; LC, local complications; ICU, intensive care unit treatment; OF, organ failure; GS, Glasgow score.

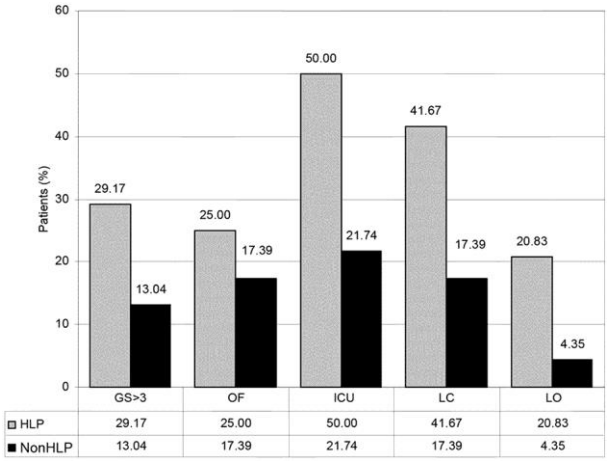

Fig 2. Correlation between lipid status and severity, course and outcome of acute pancreatitis.
HLP, patients with hyperlipidemia; NonHLP, patients without hyperlipidemia; GS, Glasgow score; OF, organ failure; ICU, intensive care unit treatment; LC, local complications; LO, lethal outcome.

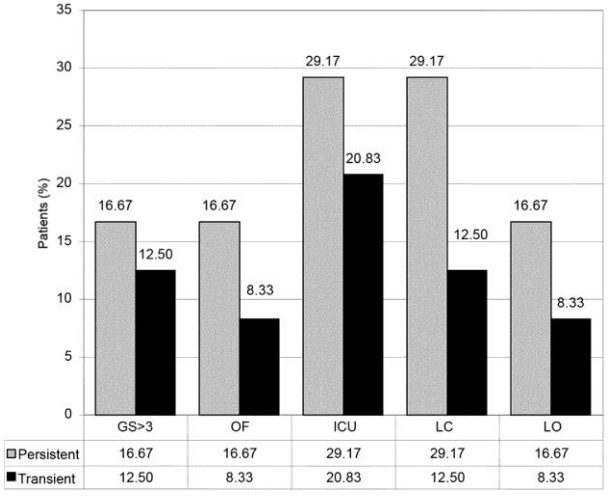

Fig. 3. The correlation between the duration of transient or persistent hyperlipidemia and severity, course and outcome of acute pancreatitis
GS, Glasgow score; OF, organ failure; ICU, intensive care unit treatment; LC, local complications; LO, lethal outcome.

Table 2. The incidence and distribution of hyperlipidemic disorders

	Hypercholesterolemia		Overall	Hypertriglyceridemia	
	Moderate	*Severe*		*Moderate*	*Severe*
Glasgow score >3	0	2	2 : 5	2	3
Organ failure	1	1	2 : 4	1	3
Intensive care unit	1	3	4 : 8	3	5
Local complications	1	3	4 : 6	2	4
Lethal outcome	0	0	0 : 5	1	4

Discussion

The results of our clinical study revealed high incidence of HLP in 51% of examined AP patients with the prevalence of severe forms in more than half of these patients. Dominant lipid disorder was HTG, followed by hypercholesterolemia. HTG is rare, but well documented cause of severe AP. In experimental model, infusion of triglycerides induces significant histopathological changes of pancreas tissue including oedema and hemorrhage, similar to AP [9]. Also, HLP deteriorates the course of experimental AP (both oedematous and necrotizing) [5, 10], while in human population with the history of AP exogenous lipids intake induces HLP with acute abdominal pain [7]. Reportedly, HTG may cause respiratory failure in patients with AP [11]. The serum triglyceride level (STL) that may induce AP or significantly influence its severity and course is not strictly determined yet. As reported by some authors, HTG-induced AP rarely develops with STL lower than 20 mmol/L [6]. Nevertheless, some reports state that STL higher than 11.3 mmol/L may initiate AP episode and that its reduction prevents further inflammation episodes [12]. It has also been reported that HTG can increase the pancreas damage during AP and predispose the transition from oedematous to necrotizing AP if STL exceeds 5.65 mmol/L [13]. STL exceeds 20 mmol/L almost exclusively in patients with genetic lipid disorder (familial HTG) and in these patients the settings of AP may induce severe pain and lead to a state called "hyperlipidemic abdominal crisis".

HTG is considered the third most frequent cause of AP, after alcohol and gallstones [6, 12]. The incidence of lipid abnormalities in AP patients varies from 3.8 to 39% [6, 7]. However, secondary lipid metabolism disorders (especially mild to moderate) are most common in alcohol-induced AP and in diabetic, pregnant and obese patients [12]. However, in spite clear correlation, it may still be difficult to differentiate between mild to moderate HLP as secondary comorbidity in AP from severe HTG that primarily induces AP, particularly in patients with hereditary HLP. Also, the influence of HLP on AP evolution is still unclear, despite well documented association between high serum triglyceride levels and some molecular mechanisms of pancreas acinar cells damage. It has been reported that HTG independently influences the severity and deteriorates necrotizing AP [14, 15]. On the other hand, some reports state that HLP does not influence the outcome of AP [2, 6, 16]. The results of our study clearly showed that patients with HLP, especially HTG, had more severe AP, higher incidence of complications and poorer outcome compared to normolipemic patients. Although this clearly indicates that HLP (HTG) worsened AP in our patients, it is reported that, in reverse, AP may induce HTG making this correlation a sort of a HTG-AP "circulus vitiosus" which may be difficult to cease [17].

Based on literature and our data from presented study, the administration of additional, specific hypolipidemic treatment (beside ordinary therapy for AP which is mandatory) would seem beneficial in patients with AP and HLP. Its goal would be maximal possible reduction of STL since it is reported that reduction of STL under 500 mg/dL [12], 1000 mg/dL [2] or 2000 mg/dL [18] may prevent abdominal pain and AP episode relapse. Although STL and serum chylomicrone levels rapidly decrease while fasting, targeted hypolipidemic therapy would accelerate plasma lipoproteins clearance. It is also well documented that dietary therapy combined with exercise and body weight loss facilitates the treatment and prevents inflammation relapse [2, 19]. The choice of hypolipidemic treatment is individual and depends on type and intensity of HLP and current disease/patient status. It includes many options, such as plasmapheresis, lipoprotein apheresis, insulin, heparin, purified apoC-II, penta-association therapy (hemofiltration and TG adsorption, statins and/or fibrates). However, despite the reasonable amount of evidence there is still a lack of agreement on routine use of these therapeutic variables. Nevertheless, many authors agree that maximal efforts should be made to prevent further hyperlipidemic AP episodes by achieving a permanent reduction of serum lipid levels using long-term medication (statins, fibrates, niacin), adequate dietary treatment (restriction of alcohol, fat and carbohydrate intake, enough antioxidants, vitamin, mineral and protein intake, fish oil supplements) and successful control of secondary aggravating factors and comorbidities (diabetes, obesity, hypothyroidism).

Conclusion

Since the incidence of HLP in patients with AP may be quite high, it should be considered and treated by a clinician as a separate serious problem, both when being a cause and a concomitant disorder. Hypolipidemic therapy should be administered both in urgent AP settings and as a long-term treatment aimed to prevent inflammation recurrence by successful persistent serum lipid levels control.

References

1. Gan SI, Edwards AL, Symonds CJ, Beck PL. Hypertriglyceridemia-induced pancreatitis: a case-based review. World J Gastroenterol 2006; 12:7197–7202.
2. Yadav D, Pitchumoni CS. Issues in hyperlipidemic pancreatitis. J Clin Gastroenterol 2003; 36:54–62.
3. Speck L. Fall von lipamia. Arch Verin Wissenschaftl Heilkunde 1865; 1:232.
4. Lin HH, Hsu CH, Chao YC. Tamoxifen-induced severe acute pancreatitis: a case report. Dig Dis Sci 2004; 49:997–999.
5. Hofbauer B, Friess H, Weber A, et al. Hyperlipaemia intensifies the course of acute oedematous and acute necrotising pancreatitis in the rat. Gut 1996; 38:753–758.
6. Fortson MR, Freedman SN, Webster PD III. Clinical assessment of hyperlipidemic pancreatitis. Am J Gastroenterol 1995; 90:2134–2139.
7. Cameron JL, Capuzzi DM, Zuidema GD, Margolis S. Acute pancreatitis with hyperlipemia: the incidence of lipid abnormalities in acute pancreatitis. Ann Surg 1973; 177:483–489.
8. Steinberg WM, Goldstein SS, Davis ND, Shamma'a J, Anderson K. Diagnostic assays in acute pancreatitis. A study of sensitivity and specificity. Ann Intern Med 1985; 102:576–580.
9. Saharia P, Margolis S, Zuidema GD, Cameron JL. Acute pancreatitis with hyperlipaemia: studies with an isolated perfused canine pancreas. Surgery 1977; 82:60–67.
10. Haig THB. Experimantal pancreatitis intensified by a high fat diet. Surg Gynecol Obstet 1970; 131:914–918.
11. Kimura T, Toung JK, Margolis S, Permutt S, Cameron JL. Respiratory failure in acute pancreatitis: a possible role for triglycerides. Ann Surg 1979; 189:509–514.
12. Toskes PP. Hyperlipidemic pancreatitis. Gastroenterol Clin North Am 1990; 19:783–791.
13. Oh RC, Lanier JB. Management of hypertriglyceridemia. Am Fam Physician 2007; 75:1365–1371.
14. Chen CH, Dai CY, Hou NJ, Chen SC, Chuang WL, Yu ML. Etiology, severity and recurrence of acute pancreatitis in southern Taiwan. J Formos Med Assoc 2006; 105:550–555.
15. Navarro S, Cubiella J, Feu F, Zambón D, Fernández-Cruz L, Ros E. [Hypertriglyceridemic acute pancreatitis. Is its clinical course different from lithiasic acute pancreatitis?], In: Abstract. Med Clin (Barc) 2004; 123:567–570. (Spanish)
16. Balachandra S, Virlos IT, King NK, Siriwardana HP, France MW, Siriwardena AK. Hyperlipidaemia and outcome in acute pancreatitis. Int J Clin Pract 2006; 60:156–159.
17. Joong HB, Sang HB, Ho SC, et al. Acute pancreatitis due to hypertriglyceridemia: report of 2 cases. Korean J Gastroenterol 2005; 46:475–480.
18. Miller A, Lees RS, McCluskey MA, Warshaw AL. The natural history and surgical significance of hyperlipemic abdominal crisis. Ann Surg 1979; 190:401–408.
19. Athyros VG, Giouleme OI, Nikolaidis NL, et al. Long-term follow-up of patients with acute hypertriglyceridemia-induced pancreatitis. J Clin Gastroenterol 2002; 34:472–475.

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EFFECTS OF SUPPLEMENTATION WITH VITAMIN E ON GENTAMYCIN-INDUCED ACUTE RENAL FAILURE IN RATS

Nenad Stojiljković[1], Sonja Ilić[1*], Milica Veljković[1], Jovan Todorović[2]

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Abstract. *A frequent administration of gentamicin in clinical practice has shown its bactericidal activity, and besides being vestibulotoxic it is highly nephrotoxic, which can further result in acute renal insufficiency. The study analyzed 24 Wistar rats, divided into three equal groups. GM group received gentamicin (100 mg/kg), GME group received vitamin E (100 mg/kg) and the same dose of gentamicin as GM rats, while the third group served as the control group and received saline (1 ml/24h) for 8 days. Pathohistological examination of the kidney tissues from GM group rats showed areas of coagulation-type necrosis in a large number of proximal tubules, while their glomeruli were considerably enlarged compared both to control and GME group rats. In GME rats, changes in glomeruli were less visible, while areas of coagulation-type necrosis were not found. Biochemical analysis showed significantly higher values of blood urea and creatinine in GM group rats in comparison to C group and GME group ($p<0.001$). The concentrations of potassium in blood serum was significantly lower in GM group compared to control group ($p<0.01$), whereas the concentration of sodium was lower, however, without statistical significance. The concentrations of AOPP for GM group were significantly higher when compared to C group ($p<0.001$), whereas the values for GME group of rats were statistically significantly lower than AOPP recorded for GM group ($p<0.001$). Our experimental study has shown that gentamicin-induced nephrotoxicity can be significantly reduced by simultaneous administration of vitamin E.*

Key words: *Gentamicin, vitamin E, nephrotoxicity, Wistar rats*

Introduction

A very frequent administration of the aminoglycoside antibiotic gentamicin in the clinical practice has shown its undoubted nephrotoxic effect [1]. Even in low concentrations, gentamicin shows its bactericidal activity, and besides being vestibulotoxic it is highly nephrotoxic, which can further result in acute renal insufficiency (ARI). Numerous experimental models have confirmed the nephrotoxicity induced by gentamicin [2–5], cyclosporine [6, 7], cisplatine [8], adriamicin [9], as well as other toxic chemical substances such as glycerol [10, 11], mercury chloride [12] and others. Nephrotoxicity induced in these experimental models showed pathohistological, ultrastructural and functional renal impairments in the form of tubular desquamation and necrosis and elevated blood urea and serum creatinine. The predilection sites of damage are the renal cortex, i.e. glomeruli and proximal tubules. In the recent years, there have been many studies pointing to the significant role of reactive oxygen species (ROS) in gentamicin-induced nephrotoxicity [13]. In their research, Sha and Schacht [14] showed that aminoglycoside antibiotics can stimulate free radical formation. Therefore, it can be claimed with great certainty that gentamicin induces ooxidative stress. Kidney cells can produce free radicals in glomerular mesangial and endothelial cells and in tubular epithelial cells [15]. Epithelial cells of proximal tubules are very sensitive to the effects of oxygen free radicals as 50% of cells die after being exposed to the effect of H_2O_2 [16]. These free radicals destroy the glomerular basement membrane, impair the tubular function, degrade the collagen and other components of matrix [17]. Because of potential gentamicin nephrotoxicity, in the recent years there has been much research on the administration of protective substances which would prevent or reduce renal alterations, and also prevent the onset and development of renal insuffuciency. Vitamin E and N-acetyl cysteine (NAC) are well known for their antioxidant activities. Vitamin E protects unsaturated fatty acids from oxydation via peroxide and other free radicals. Vitamin E and NAC have shown their protective effects in gentamicin-induced nephrotoxicity [18]. Some studies have demonstrated that vitamin E and vitamin C reduce the lipid peroxidation and increase the activities of antioxidant enzymes in diabetic rat kidneys [19]. Vitamin E pretreatment suppresses oxidative stess and glomerulosclerosis in experimental rats [20]. The administration of single doses of vitamin E has possitive effects on cisplatin-induced nephrotoxicity in rat development [21].

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The aim of the research was to show the protective effects of vitamin E on gentamicin-induced acute renal failure in rats.

Material and Methods

Twenty-four adult Wistar rats, weighing 250–300 g, were used in the present study. The animals were housed at the Institute for Biomedical Research, Faculty of Medicine in Niš. The animals were kept in polycarbonate cages under controlled conditions with the twelve-hour day/night cycle, at the temperature of 20 ° C ± 2 ° C, and the "ad libitum" access to food ("VETFARM"-Beograd) and drinking water. All experimental procedures were approved by Ethical Committee of Medical Faculty in Niš. It was documented under number 01-2625-7.

Experimental protocol

Experimental animals were randomly divided into three equal groups of 8 animals each, one of which was used as a control group. The experimental group of animals or GM group was treated with gentamicin (Galenika AD, Belgrade, Serbia) intraperitoneally in a dose of 100 mg /kg body weight (BW)/24h. The experimental group of animals treated with gentamicin and vitamin E or GME group received oily solution of vitamin E (Pharmamagist, Hungary) intraperitoneally in a dose of 100 mg/kg BW/24h and the application of the same dose of gentamicin as in the first group of rats. The control group of animals received physiological saline solution 1 ml/day intraperitoneally. All groups were treated over a period of 8 consecutive days. Following the last application, that is 9 days after the beginning of the experiment, all animals were anaesthetized using ketamine at a dose of 80 mg/kg (10% Ketamidor, Richter pharma AG, Wien, Austria). Blood samples were taken from the aorta (2ml), and the kidneys were subsequently removed.

Histological analysis

The kidneys were dissected out, washed and fixed in 10% paraformaldehyde (in 0.1M phosphate buffer saline), dehydrated in ascending graded series of alcohol and processed for paraffin embedding. Kidney tissue species were cut at a thickness of 5μm using a HistoRange microtome (model: LKB 2218, LKB-Produkter AB, Bromma, Sweden) and stained with hematoxylin–eosin (HE) for the study of morphological changes in the kidney and PAS (Periodic Acid Schiff) for verifying the content of glycogen, according to conventional staining protocols. For histopathological examination of kidney tissue the microscope (LEICA DM 2000 LED) and digital camera (LEICA DFC 450) were used.

Biochemical analysis

Blood samples were analyzed for markers of kidney function impairment. Urea, creatinine, sodium and potassium concentrations in serum were measured in the laboratory of the Department of Nephrology and Dialysis Clinical Center Niš using an automatic biochemical analyzer (A25 Biosystems, Barcelona, Spain).

Determination of protein oxidation

To determine the concentration of advanced oxidation protein products (AOPP) as a marker of oxidative modified proteins, kidney tissue was minced and homogenized in ice-water with homogenizer (IKA Works de Brasil Ltda Taquara, RJ 22, 713-00). Proteins were measured according to Lowry's method [22] using bovine serum as standard. The concentration of AOPP in the renal homogenates was determined by spectrophotometric method by Witko-Sarsat [23]. This method is based on the reaction of AOPP with potassium iodide in an acidic medium. The color intensity was recorded immediately at 340 nm. The concentrations were expressed in μmol/mg protein.

Statistical analysis

Statistical analysis of the data obtained by biochemical blood analysis were expressed as mean values and standard deviations, and statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparison (Graphpad Prism 5.03, San Diego, CA, USA).

Results

Histological analysis

In the GM group of rats, a large number of proximal tubules, especially initial convoluting portions, showed coagulation-type necrosis and apoptosis with cytoplasm vacuolation, desquamation and inflammatory cell infiltration in cells still containing nuclei. Glomeruli in this group were enlarged and paler (Fig. 1) than in the control group of animals (Figs. 2 and 3). In experimental group treated with gentamicin some of glomerular capillaries were infiltrated with neutrophil leukocytes

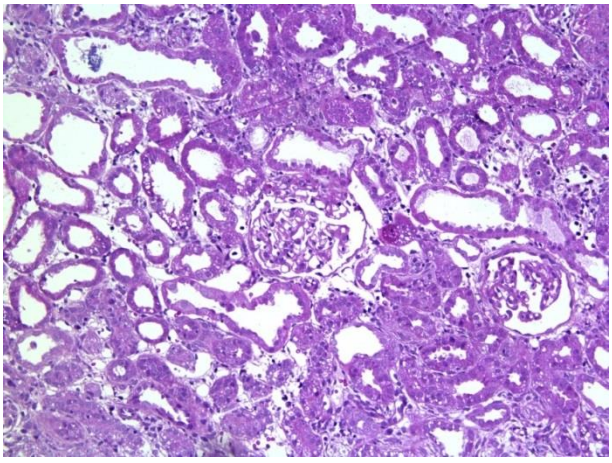

Fig. 1. Histopathological tissue features of renal glomeruli and tubules of gentamicin-treated (GM) group of rats. HE, × 200.

(Fig. 4). The distal tubules were of normal appearance. In the GME group, glomeruli were somewhat enlarged and hyaline cylinders in some proximal tubules were present; the areas of coagulation-type necrosis were not found (Figs. 5 and 6).

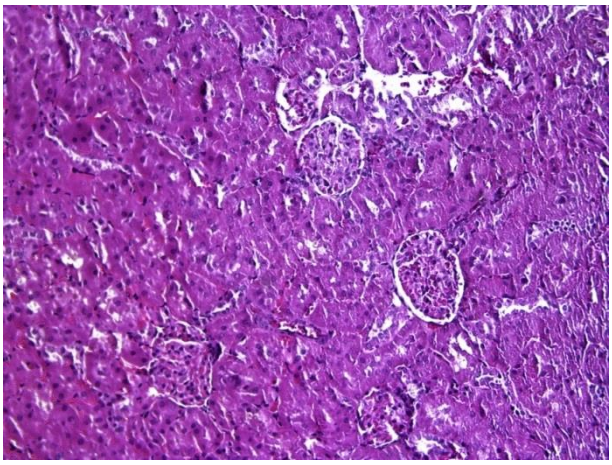

Fig. 2. Histopathological tissue features of renal glomeruli and tubules of control group of rats. HE, × 200.

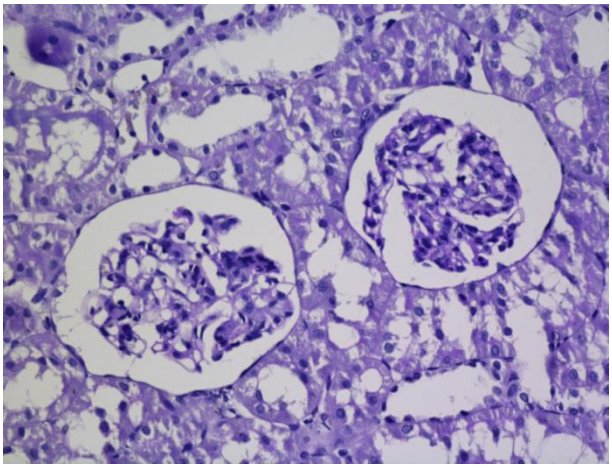

Fig. 3. Histopathological tissue features of renal glomeruli and tubules of control group of rats. PAS, × 400.

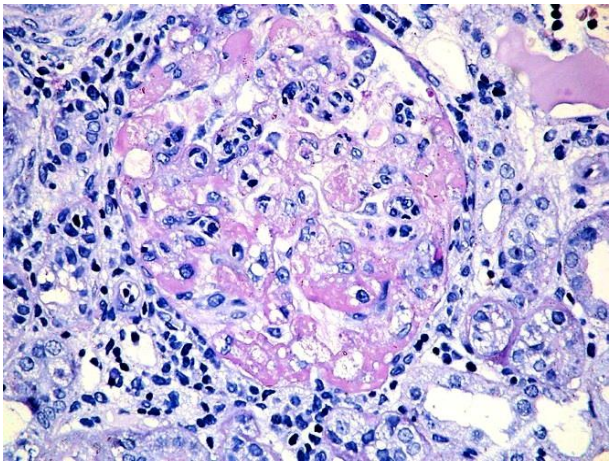

Fig. 4. Histopathological tissue features of renal glomeruli and tubules of gentamicin-treated (GM) group of rats. PAS, × 400.

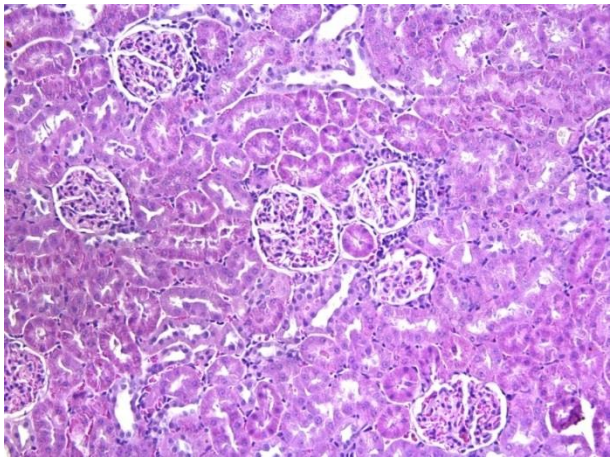

Fig. 5. Histopathological tissue features of renal glomeruli and tubules of gentamicin plus vitamin E-treated (GME) group of rats. HE, × 200.

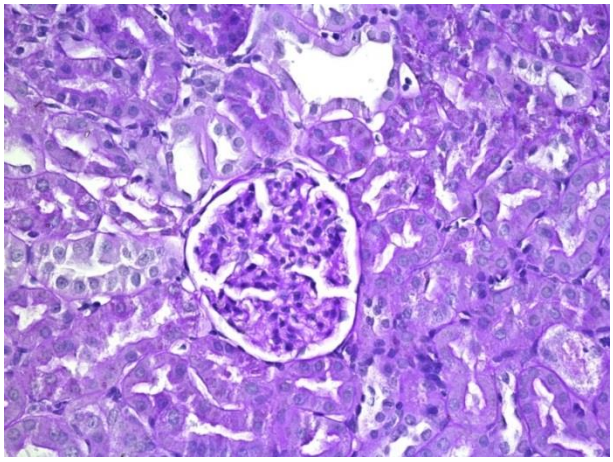

Fig. 6. Histopathological tissue features of renal glomeruli and tubules of gentamicin plus vitamin E-treated (GME) group of rats. PAS, × 400.

Biochemical analysis

Analysis of biochemical parameters showed a significant increase of urea and creatinine serum concentrations in the GM group compared to the C group ($p<0.001$). The concentration of potassium in the blood was significantly decreased in the GM group than concentration in the control group ($p<0.01$), while the concentration of sodium was lower, but not with statistical significance in comparison to the C group. In the GME group, creatinine and urea concentrations were significantly elevated compared to the control group ($p<0.01$), but also these values were significantly decreased compared to the GM group ($p<0.001$). The concentrations of potassium and sodium in the GME-group were not significantly different compared to the other groups (Table 1).

Determination of protein oxidation

Analysis of oxidative stress marker AOPP showed significantly elevated renal AOPP in the GM group than in control group of rats ($p<0.001$). Simultaneous administration of vitamin E with gentamicin reduced

oxidative stress, as evidenced by significantly decreased level of renal AOPP than those in the GM group ($p<0.001$).

Table 1. Biochemical analysis of serum levels of creatinine, urea and electrolytes in the control and experimental groups of rats

Serum concentration	C-group	GM-group	GME-group
Creatinine (μmol/L)	57.03 ±10.89	380.5 ± 29.47[#]	91.4 ± 9.987[*##]
Urea (mmol/L)	6.57 ± 0.577	30.27 ± 6.096[#]	12.73 ± 1.875[*##]
Potassium (mmol/L)	5.513± 0.5668	4.588 ± 0.4486[##]	4.988± 0.4549
Sodium (mmol/L)	143.1 ± 2.031	141.6 ± 2.2	143.6 ± 1.302

[#] $p<0.001$ vs. C-group, [##] $p<0.01$ vs. C-group, [*] $p<0.001$ vs. GM-group

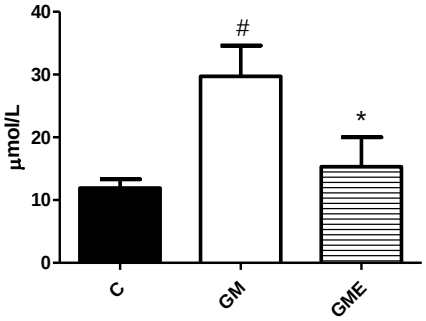

Fig. 7. Renal AOPP values in the control and experimental groups of rats.
# $p<0.001$ vs. C-group, * $p<0.001$ vs. GM-group

Discussion

Due to its strong bactericidal activity, gentamicin is a widely used antibiotic in the management of infections caused by gram-negative microorganisms. However, literature data point to its nephrotoxic effect which has been demonstrated in a large number of experimental studies in which gentamicin acute renal insifficiency was induced [24, 25].

Proximal tubule cells are severely damaged in patients treated with gentamicin or amicacin [26]. Gentamicin binds the cell wall phospholipids, blocking thus the chain reaction of phosphatidylinositol, which impairs the cell integrity [27]. The mechanism of gentamicin nephrotoxicity is complex and has not been elucidated yet. Having been administered, gentamicin reaches the renal cortex, binds the proximal tubule apical membrane, and then, by means of pinocytosis, reaches the epithelial cells of proximal tubules where it interacts with cell organelles, specifically with lysosomes and mitochondria. This further causes lysosomal destabilization, release of lysosomal enzymes and cell damage.

Pathohistological changes, confirmed by light microscopy, in our experimental group of rats treated with gentamicin (GM group) included the enlargement of glomeruli as well as the presence of neutrophil leukocytes in certain glomerular capillaries. The changes in the proximal tubules were dominant and manifested in the form of *coagulation-type necrosis*, cytoplasm vacuolization of tubular epithelial cells with preserved nuclei. The structural changes in the distal tubules were not found. These changes are mostly in keeping with the changes already described by other authors [28, 29]. In the mentioned group of animals (GM group), the biochemical analyses showed the most significant elevation of urea and creatinine levels in the serum as a sign of alterations of kidney, whereas the values of sodium did not statistically differ in all three groups of animals. The values of serum potassium in GM group were statistically significantly reduced in relation to the group of animals treated with gentamicin and vitamin E, as well as the control group. This is quite common having in mind that morphological change in the proximal tubules reduce potassium reabsorption, and consequently increases the urinary excretion of this electrolyte. Matsuda et al. [30] showed that the electrolyte composition of the renal tubular cells in gentamicin nephrotoxicity was different in relation to the necrotic and non-necrotic tubular cells of the proximal tubules. They demonstrated that histological impairment was present only in the proximal tubules, and that the concentrations of sodium and potassium in the necrotic tubular cells were somewhat lower than in the controls, whereas the concentrations of sodium in the non-necrotic cells of proximal tubules were slightly higher in relation to the control group of animals. This indicates that potassium serum levels correlate with the histopathological findings in the proximal tubule cells where gentamicin expresses its main nephrotoxic effect, which is in keeping with our results. The presence of neutrophils in the glomerular capillaries indicates that the administration of gentamicin impaired the renal microcirculation and glomerular hemodynamics. If the changes in the kidneys and glomeruli are primarily due to changed microcirculation and hemodynamics in the capillaries, the removal of these would annul gentamicin effects in renal nephrotoxicity. On the other hand, gentamicin induces oxidative stress, and achieves direct effects by means of ROS which show particular affinity for the endothelial cells of blood vessels. This induces the loss of the architectonics of the endothelial cells' cytoskeleton and organelles, impairs the cell membrane transport mechanism as well as the activity of intracellular enzyme systems. It has been proved that aminoglycoside antibiotics have harmful effects to the kidneys as they produce ROS [14]. At increased concentrations the antioxidant vitamins inhibit pathological states.

Vitamin E is the main endogenous antioxidant which reacting with oxygen radical prevents the chain reaction of free radicals, protecting thus the membrane. However, the endogenous antioxidants reserves, such as vitamin E, gradually decrease in reactions with free radicals [31]. In our experimental group of animals treated with gentamicin and vitamin E, histological and histochemical analyses of glomeruli and proximal tubules demonstrated

slightly increased glomeruli, whereas the changes on the proximal tubule epithelial cells were expressed in the form of vacuolization without any signs of necrosis. In GME group, the values of creatinine and urea were statistically significantly different in relation to the control group ($p<0.01$) and GM group ($p<0.001$) as well. The sodium and potassium serum concentrations in GM group were not statistically significantly different compared to the control group of animals. Similar to our results, the combination of vitamin E and probucol has proved to be efficient for the improvement of the renal function parameters and level of antioxidant enzymes in gentamicin-induced toxicity [32]. In other studies, a synergy between vitamin E and selenium in diminishing renal impairment has been found [3, 25]. Some studies have shown that vitamins C and E in combination provide more efficient antioxidant properties and better effects in gentamicin-induced nephrotoxicity [31]. The results of our study are in keeping with hypotheses that oxidative stress is one of the causes of gentamicin-induced renal impairment. The levels of AOPPs in the kidney homogenate of gentamicin-treated rats were significantly higher when compared with the levels of this marker in control group of animals. Witko-Sarsat et al. [23] have shown that *in vivo* AOPPs levels correlate with creatinine clearance, indicating that AOPPs are an excellent biomarker of chronic renal impairment. That AOPPs are useful biomarkers in acute renal insufficiency has been pointed out by Kimoto et al. [33] when they determined this parameter in cisplatin-induced nephrotoxicity. It is well known that vitamin E acts as an antioxidant within cells, and the mechanisms which contribute to its efficacy involve the suppression of free radicals and improvement in antioxidant system status [34]. In this paper, we confirmed the antioxidant properties of vitamin E, which was supported by statistically significantly lower AOPPs levels in rats simultaneously treated with vitamin E and gentamicin in relation to the group of rats treated with gentamicin alone.

Conclusion

Our experimental study showed that gentamicin-induced nephrotoxicity can be significantly reduced by simultaneous administration of vitamin E as a very significant antioxidant.

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References

1. Niemczyk S, Ludwicka A, Groniowski M, Lewandowski Z, Hasse Z. Nephrotoxicity of aminoglycosides. I: Preventive intraperitoneal calcium administration. Pol Arch Med Wewn 1991; 85:1–11.
2. Stojiljkovic N, Mihailovic D, Veljkovic S, Stoiljkovic M, Jovanovic I. Glomerular basement membrane alterations induced by gentamicin administration in rats. Exp Toxicol Pathol 2008; 60:69–75.
3. Ademuyiwa O, Ngaha EO, Ubah FO. Vitamin E and selenium in gentamicin nephrotoxicity. Hum Exp Toxicol 1990; 9:281–288.
4. Kavutcu A, Canbolat O, Ozturk S, et al. Reduced enzymatic antioxidant defence mechanism in kidney tissuses from gentamicin treated guinea pigs: Effects of vitamin E and C. Nephron 1996; 72:269–274.
5. Ramsammy LS, Josepovitz C, Ling KY, Lane B, Kaloyanides GJ. Failure of inhibition of lipid peroxidation by vitamin E to protect against gentamicin nephrotoxicity in the rat. Biochem Pharmacol 1987; 36:2125–2132.
6. Parra T, de Ariba G, Arribas I, Perez de Lema G, Rodriguez-Puyol D, Rodriguez-Puyol M. Cyclosporine A nephrotoxicity: role of thromboxane and reactive oxygen species. J Lab Clin Med 1998; 131:63–70.
7. Wang C, Salahudeen AK. Lipid peroxidation accompanies cyclosporine nephrotoxicity: effects of vitamin E. Kidney Int 1995; 47:927–934.
8. Luke DR, Vadiei K, Lopez-Berestein G. Role of vascular congestion in cisplatin-induced acute renal failure in the rat. Nephrol Dial Transplant 1992; 7:1–7.
9. Usta Y, Ismailoglu UB, Bakkaloglu A, et al. Effects of pentoxifylline in adriamicin-induced renal disease in rats. Pediatr Nephrol 2004; 19:840–843.
10. Luke DR, Berens KL, Verani RR. Benefit of vascular decongestion in glycerol-induced acute renal failure. Ren Fail 1991; 13:61–69.
11. Vadiei K, Brunner LJ, Luke DR. Effects of pentoxifylline in experimental acute renal failure. Kidney Int 1989; 36:466-470.
12. Vadiei K, Tucker SD, Lopez-Berestein G, Wasan KM. Nephroprotective mechanism(s) of pentoxifylline: reduction of erythrocyte-mediated vascular congestion and inhibition of nitric oxide release. Pharmacol Toxicol 1996; 78:174–180.
13. Cuzzocrea S, Mazzon E, Dugo L, et al. A role for superoxide in gentamicin-mediated nephropathy in rats. Eur J Pharmacol 2002; 450:67–76.
14. Sha SH, Schacht J. Stimulation of free radical formation by aminoglycoside antibiotics. Hear Res 1999; 128:112–118.
15. Baliga R, Ueda N, Walker PD, Shah SV. Oxidant mechanisms in toxic acute renal failure. Am J Kidney Dis 1997; 29:465–477.
16. Baud L, Hagege J, Sraer J, Rondeau E, Perez J, Ardaillou R. Reactive oxigen production by cultured rat glomerular mesangial cells during phagocytosis is associated with stimulation of lipoxigenase activity. J Exp Med 1983; 158:1836–1852.
17. Johnson JR, Lovett D, Lehrer IR, Couser GW, Klebanoff SJ. Role of oxidants and proteases in glomerular injury. Kidney Int 1994; 45:352–359.
18. Patel Manali B, Deshpande S, Shah G. Evaluation of vitamin E and N-acetyl cysteine in gentamicin-induced nephrotoxicity in rats. Ren Fail 2011; 33:341–347.
19. Kedziora-Kornatowska K, Szram S, Kornatowski T, Szadujkis-Szadurski L, Kedziora J, Bartosz G. Effects of vitamin E and vitamin C supplementation on antioxidative state and renal glomerular basement membrane thickness in diabetic kidney. Nephron Exp Nephrol 2003; 95:e134–143.
20. Hahn S, Krieg RJ Jr, Hisano S, et al. Vitamin E suppresses oxidative stress and glomerulosclerosis in rat remnant kidney. Pediatr Nephrol 1999; 13:195–198.
21. Appenroth D, Fröb S, Kersten L, Splinter FK, Winnefeld K. Protective effects of vitamin E and C on cisplatin nephrotoxicity in developing rats. Arch Toxicol 1997; 71:677–683.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folin phenol reagent. J Biol Chem 1951; 193:265–275.
23. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int 1996; 49:1304–1313.

24. Stojiljkovic N, Stoiljkovic M, Mihailovic D, et al. Beneficial effects of calcium oral coadministration in gentamicin-induced nephrotoxicity in rats. Ren Fail 2012; 34:622–627.
25. Randjelovic P, Veljkovic S, Stojiljkovic N, et al. Protective effect of selenium on gentamicin-induced oxidative stress and nephrotoxicity in rats. Drug Chem Toxicol 2012; 35:141–148.
26. Wiland P, Szechciński J. Proximal tubule damage in patients treated with gentamicin or amikacin. Pol J Pharmacol 2003; 55:631–637.
27. Walker RJ, Duggin GG. Drug nephrotoxicity. Annu Rev Pharmacol Toxicol 1988; 28:331–345.
28. Tulkens PM, Mingeot-Leclercq MP, Laurent G, Brasseur R. Conformational and biochemical analysis of the interactions between phospholipids and aminoglycoside antibiotics in relation with their toxicity. In: Brasseur R (ed) Molecular description of biological membrane components by computer-aided conformational analysis, vol II. CRC Press: Boca Raton, 1990; pp 63–93.
29. Gilbert N. Aminoglycosides. In: Mandell GL, Bennett JE, Dolin R (eds) Principles and practice of infectious disease, 5th edn. Churchill Livingstone: New York, 2000; pp 307–336.
30. Matsuda O, Beck FX, Dörge A, Thurau K. Electrolyte composition of renal tubular cells in gentamicin nephrotoxicity. Kidney Int 1988; 33:1107–1112.
31. Kadkhodaee M, Khastar H, Faghihi M, Ghaznavi R, Zahmatkesh M. Effects of co-supplementation of vitamins E and C on gentamicin-induced nephrotoxicity in rat. Exp Physiol 2005; 90:571–576.
32. Abdel-Naim AB, Abdel-Wahab MH, Attia FF. Protective effects of vitamin E and probucol against gentamicin-induced nephrotoxicity in rats. Pharmacol Res 1999; 40:183–187.
33. Kimoto Y, Sugiyama A, Nishinohara M, et al. Expressions of protein oxidation markers, dityrosine and advanced oxidation protein products in cisplatin-induced nephrotoxicity in rats. J Vet Med Sci 2011; 73:403–407.
34. Ben Amara I, Karray A, Hakim A, et al. Dimethoate induces kidney dysfunction, disrupts membrane-bound ATPases and confers cytotoxicity through DNA damage. Protective effects of vitamin E and selenium. Biol Trace Elem Res 2013; 156:230–242.

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CONNECTION OF EARLY COMPLICATIONS AFTER KIDNEY TRANSPLANTATION WITH THE GENDER OF THE RECIPIENT AND THE DONOR

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Abstract. *The frequency of vascular complications after kidney transplantation is low, but if they happen they present a great danger to the outcome of the transplantation. Renal artery thrombosis or renal vein thrombosis, as well as different types of hemorrhages can significantly jeopardize the outcome of the transplantation including the loss of the graft or mortality of the recipient. The aim of this research was to determine the connection between the frequency of early vascular complications and the gender of the recipient and the donor. The research was performed on the sample of 43 patients who underwent a kidney transplantation at the Clinic of Vascular Surgery, Clinical Centre in Niš within the period from 2009 to 2012. The difference in the representation of vascular complication between genders of the recipients (p=1) and the donors (p = 0.61) was not statistically significant. According to the results of this study, it can be concluded that the gender of the recipient and the donor is not connected to the frequency of the early vascular complications after a kidney transplantation*.

Key words: *Early vascular complications, gender, kidney transplantation*

Introduction

The frequency of vascular complications after kidney transplantation is low but if they happen they present a great danger to the outcome of the transplantation [1]. Renal artery thrombosis or renal vein thrombosis, as well as different types of hemorrhages can significantly jeopardize the outcome of the transplantation including the loss of the graft or lethality of the recipient [2]. Besides the perfection of the surgical technique, numerous vascular complications occur due to technical mistakes [3, 4].

One must also have in mind that the following illnesses of recipients in kidney transplantation are connected with some technical circumstances. Factors of comorbidity include hyper and hypocoagulable conditions, circulation paths, endothelial damage and immunosuppression [5]. The studies show that renal vein thrombosis occurs in the direct post-transplant period, usually during the first 10 days, with the presented frequency of 0.5% to 6% [6].

There are no literature data which show the connection of the gender of the recipient and the donor with early vascular complication after a kidney transplant. Starting from the assumption that the patients with a transplanted kidney stand for a high risk for developing early vascular complications, the aim of this research is to determine a connection of the frequency of early vascular complication in relation to the gender of the recipients and the donors.

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Patients and Methods

In the clinical prospective study, all patients who underwent a kidney transplantation at the Clinic of Vascular Surgery of the Clinical Centre of Niš were analysed during the period from 2009 to 2012.

The test group of patients

Patients were divided into two groups according to the type of donor:

Group A – patients who have undergone a kidney transplantation from a live related donor,
Group B – patients who have undergone a kidney transplantation from a cadaver.

Patients are divided in relation to the gender.

Early vascular complications were monitored (< 30 days after the transplantation): renal artery thrombosis; renal vein thrombosis and hemorrhage.

All the necessary data about previously mentioned test groups and vascular complications are obtained by the insight into the medical documentation of the patients (anamnesis, diagnostic procedures, surgical results, medical records and histopathological results).

Diagnostic protocol of the patients

The evaluation of live related donors was conducted on the basis of the standard diagnostic protocol of the Transplantation Center. According to the protocol, the following is necessary: complete CBC, erythrocyte sedimentation rate, basic biochemical analysis of blood,

coagulation status, haematological examination, urine sedimentation rate, urine culture, bacteriological examination, virological examination, mycological analysis, radiological examination—native ray of lungs and heart, native ray of urinary tract, intravenous pyelogram, renal arteriography, isotopic tests, blood and tissue typing, cardiorespiratory evaluation.

In all cadaveric donors, before donor nephrectomy, the following conditions had to be met: 1) period of hypotension is not longer that 30 minutes, 2) hourly diuresis is not larger than 30ml, 3) period from intubation and catheterization is not longer than 24 hours, 4) serum values of the urea and creatinin are normal.

All potential recipients were at the chronic program of hemodialysis. Evaluation and preparation for transplantations were conducted in accordance with the standard diagnostic protocol of the Center for Transplantation.

Surgical technique

The unique surgical technique for all cadaveric donors meant transperitoneal en bloc binephrectomy. Kidney preservation after donor nephrectomy was conducted by cold continuous perfusion of some of the standard perfusion solution (*Collins*, *Euro-Collins*, *Wisconsin*). Preservation of kidneys obtained from live donors was conducted *ex situ*, while preservation of kidneys obtained from cadavers was performed *in situ*. For revascularization of the graft the standard arterial anastomosis was represented by end-to-terminal anastomosis of the internal iliac with the renal artery of the graft. Termino-lateral anastomosis of the renal with the external iliac vein stood for the standard vein anastomosis. For reconstruction of the urinary tract, extraversical ureterocystoneostomy *Lich-Gregoir* was applied as a standard. In some cases where it was not possible to do an implementation of the graft by applying the aforementioned standard surgical procedures, some reconstructive urological and vascular surgical techniques were applied. According to the protocol of immunosuppressive therapy for all recipients, triple therapy which consists of cyclosporine, azathioprine and prednisone was applied. Recently, instead of azathioprine MMF has been used. During postoperative course, all patients were under constant surgical and nephrological control. By continuous monitoring of the subjective status of the patients, laboratory and clinical results, as well as by applying noninvasive and invasive extra diagnostic methods, an early diagnostics of early vascular and urological complications was conducted. For timely and adequate care of vascular and urological complications, adequate therapeutic procedures were applied.

Monitoring of the patients

Monitoring was available for all patients. The protocol of postoperative monitoring included everyday monitoring of the vital parameters, basic biochemical analysis and CBC, coagulation screening, general examination of urine, echo Doppler sonography of the allograft during the first 15 days of the postoperative period. In cases where there was a suspicion of graft rejection, a percutaneous biopsy of the graft was performed.

Monitoring was consultative with the participation of a nephrologist, urologist and vascular surgeon.

Statistical analysis

The data were analyzed by using commercial statistical The data were analyzed by using commercial statistical programs (SPSS® for Windows, v. 9.0, Chicago, USA). For comparing nonparametric data chi-square test and Fisher's exact test were used depending on the number and characteristics of features. Student's t test was used to compare parametric data if there was a normality distribution, or Mann-Whitney U test if there was no normal distribution of data. The results were presented in values ±/SD. Value $p \leq 0.05$ is considered to be statistically significant.

Results

The research was conducted on the sample of 43 patients who underwent a kidney transplant at the Clinic of Vascular Surgery of the Clinical Center in Niš during the period from 2009 to 2012. The gender structure of the recipients and donors is shown in Table 1.

Table 1. Gender structure of the recipients and donors

Parameter	Group A		Group B		Total	
	number	%	number	%	number	%
Recipients						
Male	20	55.56	3	42.86	23	55.56
Female	16	44.44	4	57.14	20	44.44
Donors						
Male	19	52.78	4	57.14	23	55.56
Female	17	47.22	3	42.86	20	44.44

NS for all parameters

The gender distribution of recipients and donors in the study group was uniform. Out of the total number of transplants, 23 transplants (53.5%) were performed on male recipients and donors and 20 transplants (46.5%) on female recipients and donors. There was no statistically significant difference between the genders in the studied groups. The age structure of the recipients is shown in Table 2.

Table 2. Age (years) structure of the recipients

Parameter	number	%	Min	Max	$\overline{X}$	SD
Group A	36	83.72	19	57	39.31	9.33
Group B	7	16.28	24	66	44.43	17.09
Total	43	100.0	19	66	40.14	10.86

p = 0.467

In relation to the total number of patients, the average age of recipients was 40.14 years, with a standard deviation of 10.86 years. The youngest recipient was 19 years old, while the oldest was 66 years. Although the average age of recipients in group B was 5.12 years higher compared to the group A, statistically significant difference between the surveyed groups was not found (p=0.467). The vascular complications in relation to the type of donors are shown in Table 3.

Table 3. Vascular complications in relation to the type of donors

Parameter	Group A		Group B		Total	
	N	%	N	%	N	%
Yes	3	8.33	1	14.29	4	9.30
No	33	91.67	6	85.71	39	90.70
Total	36	100.0	7	100.0	43	100.0

p=0.523

A total of 7 (9.3%) patients had vascular complications. There was no statistically significant relationship between groups A and B (p =0.523) for the development of early vascular complications after surgery.

The share of vascular complications in relation to the gender of the recipients is shown in Figure 1.

Observed in relation to the gender of the recipients, vascular complications occurred in 2 (8.7%) cases in males and in 2 (10%) cases in females. The difference in the representation of vascular complications between the genders was not significant (p=1).

The share of vascular complications in relation to the gender of the donors is shown in Figure 2.

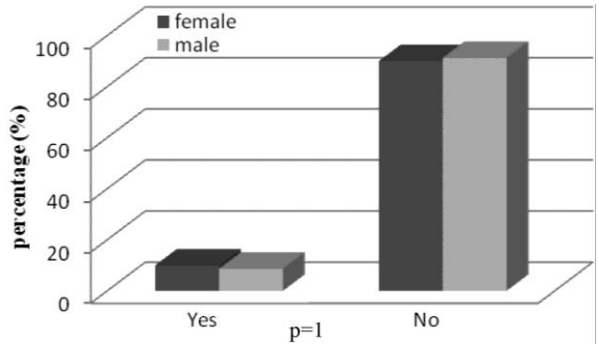

Fig. 1. Gender structure of the recipients

Observed in relation to the gender, vascular complications occurred in 3 (13.04%) cases in males and in 1 (5%) case in females. The difference in representation of vascular complications in relation to the gender of the donor is not statistically important (p = 0.61).

From total number of 43 donors, 23 (53.5 %) of them were male, while 20 (46.5 %) of them were female in which case there was no statistically significant difference between the genders.

The share of vascular complication in relation to the gender of the recipients is shown in the Figure 3.

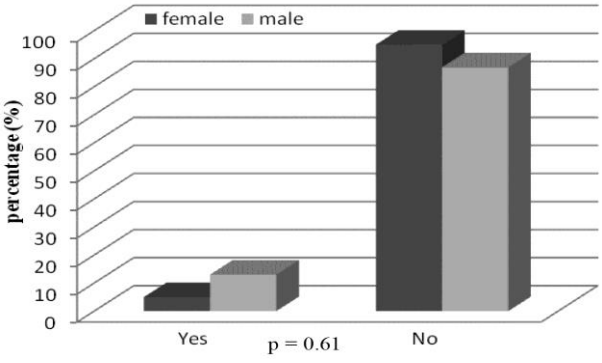

Fig. 2. Gender structure of the donors

Discussion

There are very few literature data concerning the vascular complications of kidney transplant, depending on the type of donor. Basically, all described complications are related to all of the transplanted patients, regardless of the type of donor [7]. Therefore, it is useful to consider these complications of kidney transplants from living donors and from cadavers, given the conceptual and technical differences between these two types of transplants, as well as the impact of these complications on the outcome of the procedure. The importance of the origin of the graft for certain vascular complications and the outcome of the transplant can be established by defining these complications in relation to the type of donor, as well as in relation to the applied surgical technique of implantation [8].

The benefits of transplantation from living donors include: biologically and physiologically adequate kidney, rapid social rehabilitation and reducing the number of recipients who would be waiting for cadaveric organ [9]. In our research 7 (9.3%) patients had vascular complications. Results of the research indicate the absence of statistically significant disparities between living donor and cadaveric (p =0.523) as a factor in the emergence of early vascular complications.

The average age of recipients of cadaveric group was higher by 5.12 years compared to group A. The results show that no statistically significant difference between groups of living donors and cadavers (p=0.467). Previously conducted research has shown that early transplanted vascular complications occur more frequently in kidney recipients aged 40 years and donors older than 60 years [10].

Literature data on the connection between the age of the recipient and the donor with the length of survival of the graft are different. Graft survival was longer in recipients younger than 17 years and it decreases exponentially with the age of the patient, so that the shortest was in recipients older than 60 years [11]. Research by Alexander et al. [12] showed that there was no difference in the survival of the graft donor of 56–65 years in relation to donors older than 65 years of age. At the same time donors older than 56 years had a graft survival of 10–14% less than the donor of age group16–45 years. These data indicate that donors older than 55 years had decreased renal function and detailed investigations in the selection of donors of this age has to be done [13].

There are numerous attempts to examine the influence of the gender on the outcome of the transplantation. In our examined series, male recipients were present with 53.5% and they were more numerous than female recipients who were present with 46.5%. Literature states that live donors are younger and more often female, while cadaveric donors are older and more often male [14]. In our series, live female donors were present with 53%, whereas live male donors are present with 47%. The study conducted in China shows that males and younger persons are the most common recipients, while females and middle-aged persons are the most common donors. The most common combination of live donor was mother donor son recipient [14].

In relation to the gender, vascular complications occurred in 8.7% of the cases in males and in 10% in females; there was no statistically important difference between genders (p=1). Considering the gender of the donor, vascular complications occurred in 13.04% of the cases in males and in 5% in females. The difference in representation of vascular complications in relation to the gender of the donors was also statistically insignificant (p = 0.61).

Some researches showed that there are no differences in the survival of the graft depending on the donors' gender despite the increased frequency of preformed lymphocytotoxic antibodies in female recipients [15]. Later analysis showed that the donor's gender does not affect the early postoperative outcome, but the survival of male recipients who have had the graft implanted from a female donor is shorter [16].

It is interesting that when parents are recipients of the graft from their children, there is a considerably better survival of the graft in mothers than in fathers. Some studies also showed that the survival of the graft from a male donor is longer that from a female donor [11]. The stated difference in survival increases if the donor is older than 30 years old and if cyclosporine is used in therapy. One of the possible explanations is that the nephron mass of the graft from a female donor is smaller [15].

Abou-Jaoude et al. [17] have analysed annual functionality of the graft and survival in gender combinations between donors and recipients; thus recipients and donors were divided into 4 groups: male donor- male recipient, male donor-female recipient, female donor-male recipient and female donor-female recipient. The best function of the graft was obtained in the group male donor-female recipient, while the rate of annual survival was the same in all groups [17]. On the other hand, the study by Kolonko et al. [18] showed that the combination female donor-male recipient shows a great possibility of losing the graft in 5 years. The study by McGee et al. [19] showed that in the combination of male donor-female recipient, the body mass index influences the graft survival time. Larger body mass index from male donor in relation to female recipient represents a favorable relationship [19]. Influence of metabolic parameters on the survival of the graft is more expressed in male recipients in relation to female recipients [20].

By the analysis of functional transplanted kidneys five years after surgeries Tent et al. [21] showed that there is no crucial difference between male and female donors. Transplanted kidneys adapted completely to the functional needs of the recipients regardless of their body mass index and gender [21].

It is necessary to remark that this is the first research which deals with interconnections of recipients and donors' gender with the frequency of early vascular complications. Further researches should be directed at examining the types of early vascular complications with the mentioned factors, as well as the interconnections of gender and systemic factors with the type of vascular complications.

Conclusion

The gender of the recipient is not connected with the frequency of early vascular complications after kidney transplantation.

References

1. Pillot P, Bardonnaud N, Lillaz J, et al. Risk factors for surgical complications after renal transplantation and impact on patient and graft survival. Transplant Proc 2012; 44:2803–2808.
2. Pawlicki J, Cierpka L, Król R, Ziaja J. Risk factors for early hemorrhagic and thrombotic complications after kidney transplantation. Transplant Proc 2011; 43:3013–3017.
3. Gargah T, Abidi K, Rajhi H, Ben Abdallah T, Chebil M, Lakhoua MR. Vascular complications after pediatric kidney transplantation. Tunis Med 2011; 89:458?461.
4. Ardelean A, Mandry D, Claudon M. [Vascular complications following renal transplantation: diagnostic evaluation]. J Radiol 2011; 92:343–357. (French)
5. Andrews PA, Compton F, Koffman CG, Bewick M, Chang RW. Prediction of outcome in non-heart-beating kidney transplantation. Transplant Proc 2001; 33:1121–1124.
6. Benoît G. [Surgical view of a series of 3,000 kidney transplantations]. Bull Acad Natl Med 2011; 195:351–362. (French)
7. Streeter EH, Little DM, Cranston DW, Morris PJ. The urological complications of renal transplantation: a series of 1535 patients. BJU Int 2002; 90:627–634.
8. Kälble T, Lucan M, Nicita G, et al. EAU guidelines on renal transplantation. Eur Urol 2005; 47:156–166.
9. UK Transplant. Renal transplant audit 1990-98. Bristol: UK Transplant, 2001.
10. Ilić N, Jovanović M, Basić D, Stojanović M, Stanojević G, Damnjanović Z. Correlation between early vascular posttransplant complications and the type and age of a kidney donor and a recipient. Acta Med Medianae 2014; 53:28–33.
11. Briggs JD. The recipient of renal transplant. In: Morris JP (ed). Kidney transplantation: Principles and practice, 5th edn. WB Saunders Company: Philadelphia, 2001; pp 45–59.
12. Alexander JW, Bennett LE, Breen TJ. Effect of donor age on outcome of kidney transplantation. A two-year analysis of transplants reported to the united network for organ sharing registry. Transplantation 1994; 57:871–876.
13. Alexander JW, Vaughn WK. The use of "marginal" donors for organ transplantation. The influence of donor age on outcome. Transplantation 1991; 51:135–141.
14. Liu G, Li X, Liu T, et al. Gender disparity of living donor renal transplantation in East China. Clin Transplant 2013; 27:98–103.
15. Khalifeh N, Hörl WH. Gender and living donor kidney transplantation. Wien Med Wochenschr 2011; 161:124–127.
16. Lin J, Zheng X, Xie ZL, et al. Factors potentially affecting the function of kidney grafts. Chin Med J (Engl) 2013; 126:1738–1742.
17. Abou-Jaoude MM, Abou-Jaoude WJ, Almawi WY. Sex matching plays a role in outcome of kidney transplant. Exp Clin Transplant 2012; 10:466–470.
18. Kolonko A, Chudek J, Wiecek A. Nephron underdosing as a risk factor for impaired early kidney graft function and increased graft loss during the long-term follow-up period. Transplant Proc 2013; 45:1639–1643.
19. McGee J, Magnus JH, Islam TM, et al. Donor-recipient gender and size mismatch affects graft success after kidney transplantation. J Am Coll Surg 2010; 210:718?725. e.1, 725–726.
20. Oh CK, Kim SJ, Kim JH, Shin GT, Kim HS. Influence of donor and recipient gender on early graft function after living donor kidney transplantation. Transplant Proc 2004; 36:2015–2017.
21. Tent H, Lely AT, Toering TJ, et al. Donor kidney adapts to body dimensions of recipient: no influence of donor gender on renal function after transplantation. Am J Transplant 2011; 11:2173–2180.

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PROTECTIVE EFFECT OF QUERCETIN ON CISPLATIN-INDUCED NEPHROTOXICITY IN RATS

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Abstract. *The aim of this study was to investigate the possible effect of quercetin on cisplatin-induced nephrotoxicity in rats. Experiments were done on thirty-two Wistar rats divided into four groups of 8 animals each. The CIS group received a single dose of cisplatin (8 mg/kg) intraperitoneally, whereas the CISQ group received quercetin intraperitoneally at a dose of 50 mg/kg for 9 days and a single dose of cisplatin intraperitoneally (8 mg/kg) on the fifth day. Animals in the Q group received quercetin (50 mg/kg) and the C group received saline (1 mL/day), both given intraperitoneally for 9 days. Quantitative evaluation of structural and functional alterations in the kidneys were performed by histopathological and biochemical analyses. Histological sections of kidney in CIS group revealed mild degenerative changes of proximal tubules and focal apoptosis of tubulocites, while glomeruli had reduced lobular appearance. In CISQ group these changes were ameliorated and less visible. Analysis of biochemical parameters showed significantly higher urea and creatinine serum concentrations in CIS group in comparison with C group and CISQ group ($p<0.001$). The concentrations of potassium and sodium in the CIS group were lower, but not statistically significant in comparison to the C group. Kidney MDA levels were found to be significantly higher in CIS group than those in C group ($p<0.001$), whereas the values for CISQ group were significantly lower than MDA recorded for CIS group ($p<0.001$). The results suggest that quercetin has the nephroprotective action and reduces lipid peroxidation in cisplatin-treated rats.*

Key words: *Cisplatin, quercetin, nephrotoxicity, rats*

Introduction

Cisplatin is an important chemotherapeutic agent useful in the treatment of several cancers: tumors of the testis (including extragonadal germ cell tumors), ovarian cancer, small cell and non-small cell lung cancer, squamous cell carcinomas of the head and neck. Several side effects of cisplatin have been reported, mainly nephrotoxicity and myelosuppression, that limit its clinical use [1–4]. Renal impairment begins several days after the application of cisplatin, as revealed by increases in the serum creatinine and blood urea nitrogen concentrations. The urine output is usually preserved (non-oliguric) and the urine may contain glucose and small amounts of protein, indicative of proximal tubular dysfunction [5]. The exact mechanism of action of cisplatin has not yet been fully understood. Recent studies showed that inflammation, oxidative stress injury, and apoptosis probably explain part of its nephrotoxicity. Toxic effects occur primarily in the proximal tubule, particularly in S3 segment of the tubular epithelial cells; glomeruli and distal tubules are affected subsequently [6, 7]. Substantial evidence indicates that oxidative stress is involved in renal injury secondary to cisplatin administration [8–10]. Reactive oxygen species (ROS) production, depletion of antioxidant systems and stimulation of renal accumulation of lipid peroxidation products have been listed as the main mechanisms associated with cisplatin-induced nephrotoxicity [7, 11]. That is why in recent years many authors investigated the effects of numerous antioxidants on cisplatin-induced nephrotoxicity. Flavonoids are a group of natural antioxidants, which are increasingly used in the prevention and ameliorating of these and similar pathological conditions. Flavonoids affect basic cell function such as growth, differentiation and apoptosis, because of their radical scavenging activity. Quercetin is natural flavonoid present in high concentration in fruits and vegetables like apples, onion, potatoes, broccoli, tea, soybeans, red wine. It has been shown to have very potent antioxidant and cytoprotective effects in preventing endothelial apoptosis caused by oxidants [12, 13].

The aim of this study was to investigate the protective effect of quercetin on cisplatin-induced nephrotoxicity in rats.

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Material and Methods

Thirty-two Wistar albino rats, weighing 200–250g, were used in this study. The animals were maintained under standard laboratory conditions with controlled temperature (20 ± 2 °C) and humidity (60%) with regular light cycle (12 light/12 dark). The animals were acclimatized for 1 week before the study and had free access to standard laboratory food and water ad libitum. All experimental procedures were conducted in accord with the principles for the care and use of laboratory animals in research and approved by the local ethics committee.

Experimental protocol

After a quarantine period of 7 days, 32 rats were randomly divided into four groups, each consisting of 8 animals. The control group of rats (C group) received 1 ml saline solution per day intraperitoneally for 9 days. The cisplatin group (CIS group) received a single dose of cisplatin (Pfizer Pty Ltd, Bentley, Australia) intraperitoneally on the fifth day of the experiment at a dose of 8 mg/kg. The quercetin group (Q group) was used as a positive control and received quercetin (Sigma-Aldrich, St. Louis, Missouri, USA) dissolved in physiological saline solution, intraperitoneally, at a dose of 50mg/kg for 9 days and the cisplatin-quercetin group (CISQ group) received quercetin intraperitoneally at a dose of 50 mg/kg for 9 days and cisplatin intraperitoneally on the fifth day of treatment at a dose of 8 mg/kg. Ten days after the beginning of the experiment all animals were anaesthetized using 80 mg/kg ketamine (Ketamidor 10%, Richter Pharma AG, Wels, Austria) and sacrificed. Blood samples for biochemical analysis were taken from the aorta (2 mL), and the kidney was subsequently removed and separated into two parts for biochemical analysis and light microscopic examination.

Histological analysis

Paraformaldehyde-fixed kidney tissues were dehydrated in ascending graded series of alcohol and embedded in paraffin. Kidney tissue specimens were cut into slices of 5 μm thickness using a HistoRange microtome (model: LKB 2218, LKB-Produkter AB, Bromma, Sweden) followed by staining with hematoxylin and eosin (HE) according to conventional staining protocols. The histological sections were examined with a light microscope Leica DMR (Leica Microsystems AG, Wetzlar, Germany).

Biochemical analysis

After finishing the experiment, blood samples taken from the aorta were analyzed for markers of renal impairment. Urea, creatinine, sodium and potassium concentrations in serum were measured using an automatic biochemical analyzer (A25 Biosystems, Barcelona, Spain) in the laboratory of the Department of Nephrology and Dialysis Clinical Center Niš.

Estimation of lipid peroxidation

Lipid peroxidation was measured in terms of malondialdehyde (MDA). The intensity of LPO in kidney tissue was spectrophotometrically measured based on the thiobarbituric (TBA) response products [14]. Homogenate absorption was measured at 532 nm. The malondialdehyde / lipid peroxidation end-product concentration was expressed in mg/protein using the molecular extinction coefficient of MDA (1.56×10^{-5} mol cm^{-1}).

Statistical Analysis

All data were expressed as the mean ± SD. Statistical comparison between different groups were done by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparison (Graphpad Prism version 5.03, San Diego, CA, USA). $P< 0.05$ were considered to be statistically significant.

Results

Histological analysis

In the CIS group of animals a large number of proximal tubules showed degeneration of tubular architecture, with numerous vacuoles in cytoplasm of tubular cells and focal apoptosis of tubulocytes. The distal tubules were with normal histological appearance. Glomeruli had reduced lobular appearance with hyperemia (Fig. 1). In the CISQ group these changes were less pronounced with focal degenerative changes in proximal tubules, while glomeruli were not affected (Fig. 2). Renal sections from the C and Q groups showed no histological changes (Figs. 3 and 4).

Biochemical analysis

In the CIS group, when compared to the C group, analysis of biochemical parameters showed a significant increase of urea and creatinine serum concentrations ($p <0.001$). The concentration of potassium and sodium in the CIS group were lower, but not statistically significant in comparison to the C group. In the CISQ group, creatinine concentrations were significantly elevated compared to the first group ($p <0.05$), but also these values were significantly decreased compared to the CIS group ($p<0.001$). Levels of urea were significantly elevated in CISQ group in comparison with the C group ($p<0.001$) and significantly lower when compared to the CIS group of animals ($p<0.001$). The concentrations of potassium and sodium in the CISQ group were not significantly different compared to the other groups (Table 1).

Estimation of lipid peroxidation

Cisplatin administration to rats significantly increased the MDA levels in kidney tissue compared to C group ($p<0.001$). Administration of quercetin in the CISQ group reduced lipid peroxidation, as evidenced by significantly decreased level of MDA than those in the CIS group ($p<0.001$) (Fig. 5).

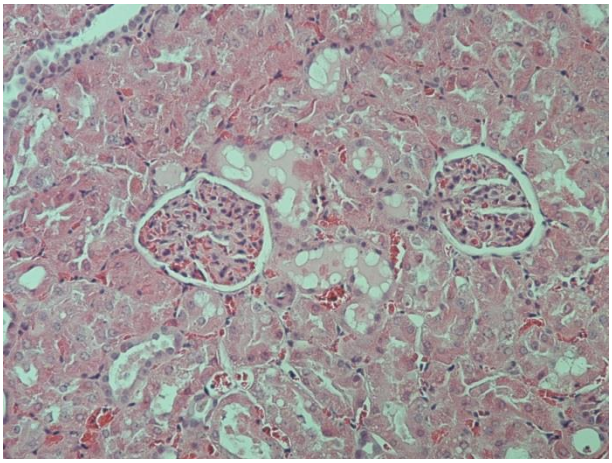


Fig. 1. Histopathological view of renal sections of CIS group of rats (HE × 200)

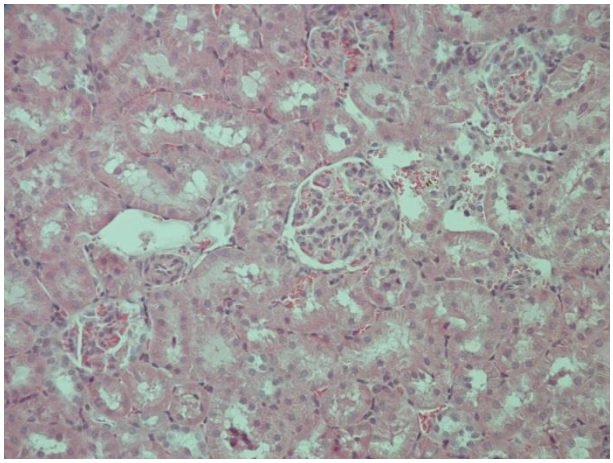


Fig. 3. Histopathological view of renal sections of C group of rats (HE × 200)

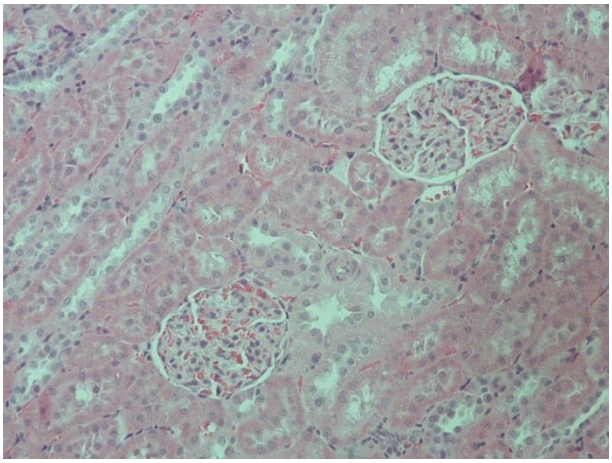


Fig. 2. Histopathological view of renal sections of CISQ group of rats (HE × 200)

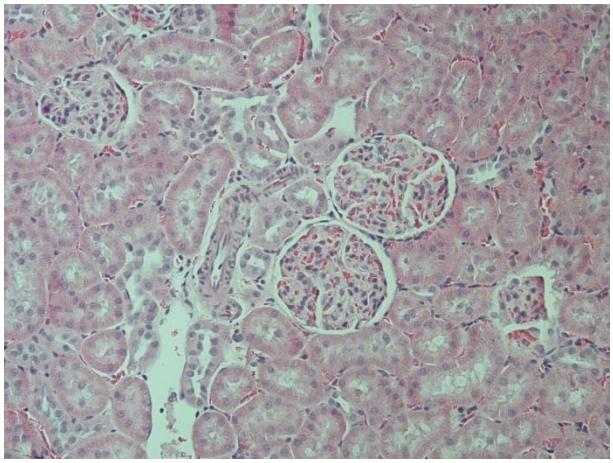
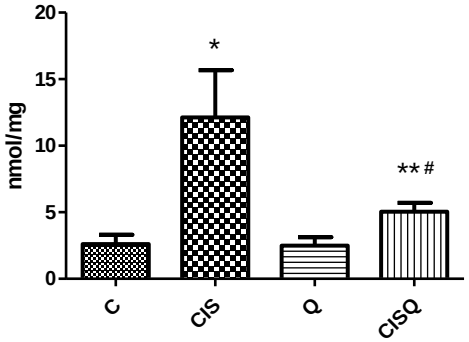


Fig. 4. Histopathological view of renal sections of Q group of rats (HE × 200)

Table 1. Biochemical analysis of rat serum creatinine, urea and electrolytes concentrations.

Serum contretation	C group	CIS group	Q group	CISQ group
Creatinine (µmol/L)	41.36 ± 5.33	268.4 ± 55.39*	46.31 ± 7.665	86.14 ± 20.57**#
Urea (mmol/L)	5.75 ± 0.8142	36.07 ± 7.059*	6.888 ± 1.625	22.03 ± 7.109*#
Sodium (mmol/L)	139.1 ± 2.167	137.5 ± 2.777	140.1 ± 2.8	138.4 ± 2.299
Potassium (mmol/L)	4.95 ± 0.3546	4.375 ± 0.6228	4.988 ± 0.6728	4.771 ± 0.3251

#p< 0.001 vs. CIS, *p< 0.001 vs. C, **p<0.05 vs. C



# p<0.001 vs. C, ** p<0.05 vs. C, # p<0.001 vs. CIS

Fig. 5. Values of kidney malondialdehyde in rats treated with cisplatin

Discussion

The anticancer drug cisplatin is a very effective compound in the treatment of several cancers. Its clinical use, however, is associated with severe side effects. Main side effect which limits its use in treatment of cancers is nephrotoxicity [1, 3, 15]. Cisplatin in the kidneys penetrates the tubular cells and reaches high concentration in the proximal tubules (S3 segment), the sites most dramatically affected [7]. Glomerular injury is less frequent. Tubular damage manifests through impaired reabsorption which is characterized by reduced glomerular filtration rate, increased serum creatinine and blood urea concentrations, hypokalemia. In 20–30% of patients treatment with

cisplatin induces acute kidney injury [5, 16]. The mechanism underlying cisplatin nephrotoxicity is incompletely defined. The pathophysiological mechanism of cisplatin-induced tubular damage is complex and involves a number of interconnected factors such as accumulation of cisplatin mediated by membrane transportation, conversion into nephrotoxins, DNA damage, mitochondrial dysfunction oxidative stress, inflammatory response, activation of signal transducers and intracellular messengers and activation of apoptotic pathways [7]. In our study, quantitative evaluation of cisplatin-induced structural alterations and degree of functional alterations in the kidneys were performed by histopathological and biochemical analyses in order to determine potential beneficial effects of quercetin on cisplatin-induced nephrotoxicity. Histopathological analyses in CIS group showed easy to moderate disturbed organization of the epithelium with mild degree of degeneration and abundant cytoplasm in the proximal tubules. There were also a large number of vacuoles in the cytoplasm of tubulocytes, as well as apoptosis of certain tubules. Distal tubules were not significantly altered. Glomeruli had changed reduced lobular appearance and pronounced hyperemia. Palipoch et al. showed that the nephrotoxicity of cisplatin is dose-dependent; administration of different doses of cisplatin caused various degrees of renal impairment. At the dose of 10 mg/kg cisplatin caused presence of proteinaceous casts in the tubular lumen. Higher doses caused mild to moderate tubular necrosis, especially in the proximal tubules, in the same study. In our experiments, rats treated with quercetin revealed an almost complete prevention of histopathological alterations. There were focal degenerative changes in proximal tubules, without vacuoles and apoptosis, while glomeruli were not affected. These findings are in agreement with earlier reports [18–20] where histological changes were also consistent with laboratory findings. We found that a single dose of cisplatin caused significant increase of urea and creatinine serum concentrations ($p < 0.001$) compared with control group. The concentrations of potassium and sodium in the CIS group were lower, but not statistically significant in comparison to the C group. The toxic effects of cisplatin in our study were similar to those shown by Badary et al., who caused nephrotoxicity by cisplatin administration (7 mg/kg) as evidenced by significantly increased levels of urea and creatinine levels compared with control group. We showed that administration of quercetin (50 mg/kg), 4 days before and 4 days after single dose of cisplatin had beneficial effect on kidney treated with cisplatin. This protection was evidenced by significantly reduced levels of urea and creatinine in CISQ group in relation to the group of animals that received only cisplatin. These findings are in accordance with the results described previously [18, 19, 22]. Shimeda et al. [23] and Koyner et al. [24] suggested that underlying mechanism in cisplatin-induced nephrotoxicity is oxidative stress through elevation of ROS and reduction of the antioxidant defense system. Cisplatin generates ROS such as superoxide anion and hydroxyl radicals and stimulates renal lipid peroxidation [25–27]. It has been shown in various studies that cisplatin administrations are associated with increased formation of free radicals, and with heavy oxidative stress [26, 28, 29]. As a marker of oxidative stress and lipid peroxidation we evaluated MDA in kidneys. Levels of MDA were significantly increased in rats treated with cisplatin when compared to the control group probably due to impairment of antioxidant system. However, lipid peroxidation was significantly reduced in the animals that received cisplatin and quercetin compared with CIS group. Quercetin significantly attenuated the increase of MDA levels in renal tissue probably because of its capacity to scavenge oxygen free radicals in the kidney tubular cells of rats. Many studies suggested that quercetin has a broad range of pharmacological activities, such as anticancer, antioxidant and anti-inflammatory [30–32]. In addition to its protective effects, quercetin alone was found to be safe and did not induce any histopathological or biochemical changes in the kidneys.

Conclusion

The findings in our study clearly showed that quercetin ameliorated oxidative and histological damage caused by cisplatin. These results may indicate that quercetin is beneficial as a protective agent in cisplatin-induced nephrotoxicity.

References

1. Mansour MA, Mostafa AM, Nagi MN, Khattab MM, Al-Shabanah OA. Protective effect of aminoguanidine against nephrotoxicity induced by cisplatin in normal rats. Comp Biochem Physiol Part C 2002; 132:123–128.
2. Von Hoff DD, Schilsky R, Reichert CM, et al. Toxic effects of cis-dichlorodiammineplatinum(II) in man. Cancer Treat Rep 1979; 63:1527–1531.
3. Goldstein RS, Mayor GH. Minireview. The nephrotoxicity of cisplatin. Life Sci 1983; 32:685–690.
4. Florea AM, Büsselberg D. Cisplatin as an anti-tumor drug: cellular mechanisms of activity, drug resistance and induced side effects. Cancers 2011; 3:1351–1371.
5. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of cisplatin nephrotoxicity. Toxins 2010; 2:2490–2518.
6. Chirino YI, Pedraza-Chaverri J. Role of oxidative and nitrosative stress in cisplatin-induced nephrotoxicity. Exp Toxicol Pathol 2009; 61:223–242.
7. Peres LA, da Cunha AD Jr. Acute nephrotoxicity of cisplatin: molecular mechanisms. J Bras Nefrol 2013; 35:332–340.
8. Ma Z, Wei X, Fontanilla C, et al. Caffeic acid phenethyl ester blocks free radical generation and 6-hydroxydopamine-induced neurotoxicity. Life Sci 2006; 79:1307–1311.
9. Santos NA, Catão CS, Martins NM, Curti C, Bianchi ML, Santos AC. Cisplatin induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Arch Toxicol 2007; 81:495–504.

10. Cetin R, Devrim E, Kiliço?lu B, Avci A, Candir O, Durak I. Cisplatin impairs antioxidant system and causes oxidation in rat kidney tissues: possible protective roles of natural antioxidant foods. J Appl Toxicol 2006; 26:42–46.
11. Yao X, Panichpisal K, Kurtzman N, Nugent K. Cisplatin nephrotoxicity: a review. Am J Med Sci 2007; 334:115–124.
12. Renugadevi J, Prabu SM. Quercetin protects against oxidative stress-related renal dysfunction by cadmium in rats. Exp Toxicol Pathol 2010; 62:471–481.
13. Choi YJ, Kang JS, Park JH, Lee YJ, Choi JS, Kang YH. Polyphenolic flavonoids differ in their antiapoptotic efficacy in hydrogen peroxide-treated human vascular endothelial cells. J Nutr 2003; 133:985–991.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95:351–358.
15. Pabla N, Dong Z. Cisplatin nephrotoxicity: mechanisms and renoprotective strategies. Kidney Int 2008; 73:994–1007.
16. Hanigan MH, Devarajan P. Cisplatin nephrotoxicity: molecular mechanisms. Cancer Ther 2003; 1:47–61.
17. Palipoch S, Punsawad C. Biochemical and histological study of rat liver and kidney injury induced by Cisplatin. J Toxicol Pathol 2013; 26:293–299.
18. Sanchez-Gonzalez PD, Lopez-Hernandez FJ, Perez-Barriocanal F, Morales AI, Lopez-Novoa JM. Quercetin reduces cisplatin nephrotoxicity in rats without compromising its anti-tumour activity. Nephrol Dial Transplant 2011; 26:3484–3495.
19. Behling EB, Sendão MC, Francescato HD, Antunes LM, Costa RS, Bianchi Mde L. Comparative study of multiple dosage of quercetin against cisplatin-induced nephrotoxicity and oxidative stress in rat kidneys. Pharmacol Rep 2006; 58:526–532.
20. Arjumand W, Seth A, Sultana S. Rutin attenuates cisplatin induced renal inflammation and apoptosis by reducing NF?B, TNF-? and caspase-3 expression in Wistar rats. Food Chem Toxicol 2011; 49:2013–2021.
21. Badary OA, Abdel-Maksoud S, Ahmed WA, Owieda GH. Naringenin attenuates cisplatin nephrotoxicity in rats. Life Sci 2005; 76:2125–2135.
22. Kusumoto M, Kamobayashi H, Sato D, et al. Alleviation of cisplatin-induced acute kidney injury using phytochemical polyphenols is accompanied by reduced accumulation of indoxyl sulfate in rats. Clin Exp Nephrol 2011; 15:820–830.
23. Shimeda Y, Hirotani Y, Akimoto Y, et al. Protective effects of capsaicin against cisplatin-induced nephrotoxicity in rats. Biol Pharm Bull 2005; 28:1635–1638.
24. Koyner JL, Sher Ali R, Murray PT. Antioxidants. Do they have a place in the prevention or therapy of acute kidney injury? Nephron Exp Nephrol 2008; 109:109–117.
25. Halliwell B, Gutteridge JMC. Lipid peroxidation: a radical chain reaction. In: Halliwell B, Gutteridge JMC (eds) Free radicals in biology medicine. Clarendon Press: Oxford, 1999; pp 188–276.
26. Weijl NI, Cleton FJ, Osanto S. Free radicals and antioxidants in chemotherapy-induced toxicity. Cancer Treat Rev 1997; 23: 209–240.
27. Taguchi T, Nazneen A, Abid MR, Razzaque MS. Cisplatin-associated nephrotoxicity and pathological events. Contrib Nephrol 2005; 148:107–121.
28. Antunes LM, Darin JD, Bianchi MD. Protective effects of vitamin c against cisplatin-induced nephrotoxicity and lipid peroxidationin adult rats: a dose-dependent study. Pharmacol Res 2000; 41:405-411.
29. Antunes LM, Darin JD, Bianchi Nde L. Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. Pharmacol Res 2001; 43:145–150.
30. Bischoff SC. Quercetin: potentials in the prevention and therapy of disease. Curr Opin Clin Nutr Metab Care 2008; 11:733–740.
31. Ciftci O, Ozdemir I, Vardi N, Beytur A, Oguz F. Ameliorating effects of quercetin and chrysin on 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced nephrotoxicity in rats. Toxicol Ind Health 2012; 28:947–954.
32. Conklin KA. Dietary antioxidants during cancer chemotherapy: impact on chemotherapeutic effectiveness and development of side effects. Nutr Cancer 2000; 37:1–18.

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TAXONOMIC AND PHARMACOLOGICAL VALORIZATION OF THE MEDICINAL FLORA IN SVRLJIŠKI TIMOK GORGE (EASTERN SERBIA)

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Abstract. *This paper presents the results of taxonomic and pharmacological valorization of autochthonous medicinal flora in the Svrljiški Timok gorge in Eastern Serbia. The taxonomic structure of group of medicinal plants in the study area was compared with spectrums of medicinal flora of Serbia and similar geographic objects in the region. Herbal substances are listed and the main effect and safety of use is provided for each substance. After the discussion on threat status for each species, necessity of protection of this gorge, characterized by pronounced species diversity and richness in resources of medicinal plants, was specified.*

Key words: *Pharmacological valorization, medicinal plants, herbal substances*

Introduction

The term "medicinal plants" designates plants containing pharmacologically active substances, where dried and fresh parts are either directly used in therapy or further processed in order to derive medicinal substances or phyto-preparations [1]. Many medicinal plant species are important as source material for industrial production of medicines and various dietetic, cosmetic, hygienic and other plant products, widely used at the global scale. Due to the high concentration of biologically active substances, they have an important role both in traditional and in modern medicine (phytotherapy). Use of plants for medicinal purposes is an integral part of medicine and these medicaments and therapeutic methods are highly important, while in certain conditions, particularly regarding the chronic and the less severe ailments, they may completely substitute for other, more expensive medicaments [2]. Use of medicinal herb preparations is increasing throughout the world and it is assumed that the amount of products in the market will keep increasing as well.

Of the 3662 taxa presently known to comprise the vascular flora of Serbia [3] the group of medicinal plants includes 420 species [2]. Sporadic data on use of medicinal plants in Serbia have been recorded since the 14th century [2], while in the 19th and 20th century there was an expansion in research, primarily marked by appearance of several monographic works listing species with known medicinal properties. Dr. Josif Pančić [4, 5] was the first to provide data on medicinal flora of Serbia and use of medicinal plants. Contribution by other authors [6, 7] is also highly important, as they greatly popularized the use of medicinal plants in Serbia, offering both data on use of medicinal plants and on their distribution and habitats. Research activity was intensified in the second half of 20th century [8], introducing a modern approach to describing the current conditions and distribution of medicinal plants in Serbia, based both on experience of folk medicine and the laboratory research.

However, the modern studies of medicinal flora in specific geographic units in Serbia are still ongoing and therefore insufficient for rationalization of use of this natural resource [9]. While most of the studied floras pertain to mountain areas or certain administrative regions [9–15], the importance of gorges and canyons in Serbia, acknowledged as particularly important areas in terms of richness and conservation of flora, is still not sufficiently recognized.

The role of refugium gorges and canyons of Balkan Peninsula stems from the high level of richness, originality and ancient character of their flora [16]. The goal of this paper is to present the list of recorded plant species and results of analysis of the taxonomic structure of medicinal flora in the wider area of Svrljiški Timok gorge. It combines the list of recorded medicinal plants with the diversity of their most important medical uses and forms. In this era of mass exploitation of natural resources, it was also necessary to include an overview on the aspect of threat status and conservation of these species, as their collecting is a potential threat factor to populations and habitats of autochthonous medicinal flora in the study area.

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The gorge of Svrljiški Timok is situated in Eastern Serbia, occupying a 15 km long part of river valley of Timok between Svrljiška and Knjaževačka Kotlina valleys. The gorge is in a hill region, mostly composed of massive limestone rocks. According to altitude, exposition and type of atmospheric circulations, the gorge may be assigned to Moravica-Svrljig climatological region, with partial Mediterranean influences in the pluviometric regime [17]. In contrast to most other gorges and canyons of Eastern Serbia, which are either completely or partially in W-E direction and have one less insulated cliff, the plant life of this gorge has a more thermophilous character [16] as the main direction of this gorge is N-S. In spite of high vertical cliffs above the river, this gorge is mostly open, enabling relatively good insulation during most of the day, strongly influencing the habitat character, vegetation type and composition of flora.

The wider area of the gorge is within the zone of forest vegetation dominated by the climatogeneous forest of Italian and Turkey Oak with hornbeam *(Quercetum farnetto-cerris* subass. *carpinetosum orientalis*). Steep rocky slopes are inhabited by low hornbeam forests and scrub *(Carpinetum orientalis),* alternating with lilac-hornbeam scrub (*Syringo-Carpinetum orientalis*). According to Mišić [16], fragments of polydominant forests (*Carpino orientalis-Quercetum mixtum* and *Syringo-Aceri monspessulani-Coryletum colurnae*) develop at the more sheltered places, as a specific feature of refugium habitats in gorges and canyons of Balkan Peninsula. The eroded slopes of the gorge are overgrown with xerophilous types of herbaceous vegetation (pastures, meadows and stony ground) and diverse vegetation of fissures in limestone rocks.

Material and Methods

The size of study area is app. 66 km^2, including the NW slopes of Mt. Tresibaba and the extreme SE slopes of Mt. Devica, within the squares EP81, EP91 and EP92 (UTM system, zone 34T). The field studies were performed from 2005 to 2008, including various aspects of the vegetation season. The collected plant material has been stored in the herbarium of the Faculty of Science and Mathematics, at the Department of Biology and Ecology of University in Niš (HMN).

The nomenclature and classification of taxa was matched with the Euro+MedChecklist [18] and regional floras [19, 20] used for identification of material. Accepting the modern definition and standpoint of expert organizations [21] on elimination of term „herbal drug" from official use, in the further text it is replaced with the term „herbal substance".

The comprehensive list of medicinal plants, based on field and literature records, was adjusted according to compendium of medicinal plants given for the country by Sarić [2] and other literature relevant for territory of Serbia [1, 22]. Table 1 includes a list of all taxa with main data - name of plant species, family, vernacular name, list of herbal substances derived from that species, main effects and uses, presence of taxa in certain pharmacopeias [23–31]. The main uses and effects, as well as chemical names of herbal substances derived from medicinal plants in this area, are presented according to the relevant literature sources [1, 31].

The conservation status of taxa was matched with the list of CITES convention and the Ordinance on strictly protected and protected wild species of plants, animals and fungi, based on Law on nature protection in Republic of Serbia (Official Gazette of the Republic Serbia, No. 36/09).

Results

The taxonomic analysis has shown that the flora of medicinal plants at Svrljiški Timok includes 190 taxa (182 species and 8 subspecies) from 53 families and 139 genera (Table 1), pertaining to more than 45 percent of the total number of assumed medicinal taxa in the flora of Serbia. From the taxonomic standpoint, the group of medicinal plants is absolutely dominated by dicotyledonous angiosperms (174 taxa, 91.6%), while monocots represent a much smaller percentage (10 taxa, 5.3%) of medicinal flora of this gorge. The ferns are represented with just 5 species (including 1 species of horsetails) and contributed to total list of medicinal flora with just 2.6%, while the group of gymnosperms is represented with a single species and a very modest share of 0.5% in the total medicinal flora of the gorge. Within the flora of Svrljiški Timok, following families were represented by the greatest number of medicinal taxa: *Labiatae* (32 taxa, 16.8%), *Compositae* (20 taxa, 10.5%), *Rosaceae* (14 taxa, 7.4%), *Leguminosae* (9 taxa, 4.7%), *Cruciferae* (8 taxa, 4.2%), *Scrophulariaceae* (8 taxa, 4.2%). The genera of medicinal plants with the highest number of species are *Acer*, *Artemisia*, *Quercus*, *Rumex*, *Salix*, *Stachys* and *Thymus*.

There are relevant data on medical use for a relatively high percentage (65.3%) of plants in the study area that are considered to be medicinal plants. Cumulatively they may be used as a biological source in preparation of 146 different herbal substances listed in the table 1, potentially being implemented in treatment of more than 90 ailments and medical disorders. The flora of this gorge is primarily a significant resource for raw herbal material produced from aerial plant organs (67%). The most important herbal substance is the upper part of flowering plant (*herba*) with 42%, followed by leaf (*folium*) with 16%, flowers and inflorescences (*flos*) with 9% of the total number of herbal substances. The herbal substances derived from processed roots and rhizomes (*radix et rhizoma*) and other underground parts of plants are represented with 18%. The suggested use of most herbal substances listed in the table is in treating ailments and disorders of digestive system (25%), followed by cardiovascular (15%), respiratory (14%), immune (12%) and skin system (9%).

In the area of Svrljiški Timok gorge there are 49 species protected by Ordinance on protected and strictly protected wild species of plants animals and fungi at national level, and 3 species protected by international CITES convention.

Table 1. List of medicinal plant taxa (species and subspecies) from Svrljiški Timok gorge

Botanical taxa and family	Vernacular name	Herbal substance	Effects and use	Presence in pharmacopoeias and other relevant literature	Conserva--tion aspect
Acer campestre L. (*Aceraceae*)	Klen	*Aceris sirupus*	Same as *A. platanoides.*		
Acer monspessulanum L. (*Aceraceae*)	Maklen	*Aceris sirupus*	Same as *A. platanoides.*		
Acer platanoides L. (*Aceraceae*)	Mleč	*Aceris sirupus*	Laxative.		
Acer tataricum L. (*Aceraceae*)	Žešlja	*Aceris cortex, A. folium.*	Astrigent.		
Achillea crithmifolia Waldst. & Kit. (*Compositae*)	Hajdučka trava	*Millefolii herba*	Same as *A. millefolium.*		
Achillea millefolium L. (*Compositae*)	Hajdučka trava	*Millefolii herba*	Cholagogue, hemostatic, dyspepsia, diarrhea.	Ph Eur, BP, PDR, E+, WHO	3
Adonis flammea Jacq. (*Ranunculaceae*)	Bulka	*Adonidis herba*	Cardiotonic, diuretic.		
Adonis vernalis L. *(Ranunculaceae)*	Gorocvet	*Adonidis herba*	Cardiotonic, diuretic.	PDR, E+	1, 2
Aegopodium podagraria L. (*Umbelliferae*)	Sedmolist	*Podagrariae herba*	Rheumatism, gout, sciatica, cold. Externally, for inflammation of the skin and hemorrhoids.	PDR	
Agrimonia eupatoria Ledeb. subsp. *grandis* (Andrz. ex Ascherson & Graebner) Bornm. (*Rosaceae*)	Petrovac	*Agrimoniae herba*	Cholagogue, diuretic, diarrhea.	Ph Eur, BP, PDR, E+	2
Ajuga reptans L. (*Labiatae*)	Puzava ivica	*Ajugae herba*	Cholelithiasis, stomach disorders.	PDR	
Alliaria petiolata (Bieb.) Cavara & Grande (*Cruciferae*)	Lukovac	*Alliariae folium recens, A. herba*	Diaphoretic, diuretic.		
Althaea officinalis L. (*Malvaceae*)	Beli slez	*Althaeae radix, A. folium*	Antitussive, mucilaginous, catarrh of the respiratory tract, bronchitis, enteritis, cystitis, urethritis, varicosities.	Ph Eur, PDR, BP, E+, WHO	3
Anacamptis morio (L.) R. M. Bateman, Pridgeon & M. W. Chase (*Orchidaceae*)	Kaćun	*Salep tuber*	Same as *O. simia.*	PDR	1, 3
Anacamptis pyramidalis (L.) L. C. M. Richard (*Orchidaceae*)	Plaštak	*Salep tuber*	Same as *O. simia.*		1, 3
Anchusa officinalis L. (*Boraginaceae*)	Volovski jezik	*Anchusae herba, A. flos*	Mild diuretic, expectorant.		
Anthemis arvensis L. (*Compositae*)	Prstenak	*Anthemidis (arvensis) flos*	Carminative, dyspepsia.		
Anthyllis vulneraria L. subsp. *polyphylla* (DC.) Nyman (*Leguminosae*)	Belodun	*Anthyllidis vulnerariae flores*	Dermatic, diuretic, depurative.	PDR	3
Arctium lappa L. subsp. *lappa* (*Compositae*)	Čičak	*Bardanae radix*	Anorexia, gout, rheumatoid cystitis. Externally, for eczema and psoriasis.	PDR, E-	3
Arctium tomentosum Miller (*Compositae*)	Čičak veliki maljavi	*Bardanae radix*	Same as *A. lappa.*	E-	
Artemisia absinthium L. (*Compositae*)	Pelen	*Absinthii herba*	Roborantium, cholagogue, carminative.	Ph Eur, BP, PDR, E+	
Artemisia alba Turra (*Compositae*)	Rudinski pelin	*Artemisiae (albae) herba*	Similar as *A. absinthium.*		
Artemisia pontica L. (*Compositae*)	Sitan pelin	*Absinthii pontici herba*	Similar as *A. absinthium.*		3
Artemisia vulgaris L. (*Compositae*)	Crna komonika	*Artemisiae (vulgaris) herba*	Antimicrobial, dyspepsia.	PDR, E-	
Asparagus officinalis L. (*Asparagaceae*)	Špargla	*Asparagi herba, A. rhizoma*	Slight diuretic (herba), infections of urinary tract, kidney stones (rhizoma).	PDR, E+ (rhiz), E- (herb)	

Asplenium ceterach L. (*Polypodiaceae*)	Zlatna paprat	*Ceterach folium*	Diuretic, astrigent, diarrhea.		
Asplenium scolopendrium L. (*Polypodiaceae*)	Jelenji jezik	*Scolopendrii folium*	Diuretic, mild laxative, kidney stones.	PDR	
Ballota nigra L. (*Labiatae*)	Modri tetrljan	*Ballotae herba*	Anthelmintic, mild sedative.	BP, PDR	
Bellis perennis L. (*Compositae*)	Bela rada	*Bellidis perennis herba*	Astrigent, antiphlogistine, expectorant.	PDR	
Berberis vulgaris L. (*Berberidaceae*)	Žutika	*Berberidis cortex radicis*	Stomachic, tonic, cholagogue.	PDR, E-	
Bidens tripartitus L. (*Compositae*)	Kozji rogovi	*Bidentis herba*	Astrigent, diuretic.	PDR, WHO	
Brassica nigra (L.) Koch (*Cruciferae*)	Crna slačica	*Sinapis nigrae semen*	Rubefacient. In folk medicine as antirheumatic, against neuritis, pleurisy and acute bronchitis.	PDR	
Campanula rapunculus L. (*Campanulaceae*)	Zijevčica	*Campanulae radix, C. herba*	Wounds healing.		
Campanula trachelium L. (*Campanulaceae*)	Zvončić brazdasti	*Campanulae radix, C. herba*	Similar as *C. rapunculus.*		
Capsella bursa-pastoris (L.) Medicus (*Cruciferae*)	Tarčužak	*Bursae pastoris herba*	Hemostatic, diarrhea.	PDR, E+	
Cardamine impatiens L. (*Cruciferae*)	Režuha	*Cardamine folium*	Against chronic catarrh of the respiratory tract.		
Centaurea jacea L. (*Compositae*)	Vasiljak	*Centaureae jaceae flos sine calycibus*	Diuretic, cholagogue.		
Centaurium erythraea Rafin. (*Gentianaceae*)	Kičica	*Centaurii herba*	For appetite, anorexia, chronic dyspepsia.	Ph Eur, BP, PDR, E+	3
Chelidonium majus L. (*Papaveraceae*)	Rusa	*Chelidonii herba*	Externally, for warts, eczema and psoriasis.	Ph Eur, BP, PDR, E+, WHO	
Cichorium intybus L. (*Compositae*)	Ženetrga	*Cichorii herba et radix*	Cholagogue, diuretic, for appetite, dyspepsia.	PDR, E+	
Clinopodium menthifolium Merino (*Labiatae*)	Divlja metvica	*Calaminthae officinalis herba*	Mild sedative, tonic.		
Colchicum autumnale L. (*Colchicaceae*)	Mrazovac	*Colchici semen*	Antiphlogistine, gout, skin cancer.	PDR, E+	3
Cornus mas L. (*Cornaceae*)	Dren	*Corni fructus*	Astrigent, tonic, diarrhea.		3
Corylus avellana L. (*Corylaceae*)	Leska	*Coryli avellanae folium, C. a. cortex, C. a. semen*	Diarrhea.		3
Corylus colurna L. (*Corylaceae*)	Medveđa leska	*Coryli colurnae folium, C. c. cortex, C. c. semen*	Similar as *C. avellana.*		3
Cotinus coggygria Scop. (*Anacardiaceae*)	Ruj	*Cotini folium*	Astringent, hemostatic.		
Crataegus monogyna Jacq. (*Rosaceae*)	Beli glog	*Crataegi folium cum flore*	Cardiotonic, hypotensive, antiarrhythmic, sedative.	Ph Eur, BP, E+, WHO	3
Cynoglossum officinale L. (*Boraginaceae*)	Mišinac	*Cynoglossi folium, C herba, C. radix*	Burns, ulcers, edema.	PDR, E-	
Datura stramonium L. (*Solanaceae*)	Tatula	*Stramonii folium*	Antispasmodic.	Ph Eur, BP, PDR, E-	
Daucus carota L. (*Umbelliferae*)	Šargarepa	*Dauci radix recens, D. fructus*	Digestive, antidiabetic, antispasmodic (fructus).	PDR	
Descurainia sophia (L.) Webb ex Prantl (*Cruciferae*)	Strižica	*Descurainiae folium recens, D. herba*	Wounds healing, expectorant.		
Dictamnus albus L. (*Rutaceae*)	Jasenak	*Dictamni radix*	Anthelmintic.	PDR	
Digitalis ferruginea L. (*Scrophulariaceae*)	Besniče	*Digitalis lanatae folium*	Cardiotonic, indirect diuretic.	PDR	
Digitalis grandiflora Miller (*Scrophulariaceae*)	Krupnocvetni naprstak	*Digitalis grandiflorae folium*	Similar as *D. lanata*		
Digitalis lanata Ehrh. (*Scrophulariaceae*)	Besniče	*Digitalis lanatae folium*	Cardiotonic, indirect diuretic.	PDR	
Dryopteris filix-mas (L.) Schott (*Polypodiaceae*)	Navala	*Filicis maris rhizoma*	Anthelmintic, migraines.	PDR, E-	
Epilobium angustifolium L. (*Onagraceae*)	Noćurak	*Epilobii herba (folium)*	BHP.	PDR	

Equisetum arvense L. (*Equisetaceae*)	Poljski rastavić	*Equiseti herba*	Diuretic.	Ph Eur, BP, PDR, E+, WHO	3
Erodium cicutarium (L) L'Her. (*Geraniaceae*)	Živa trava	*Erodii cicutarii herba*	Hemostatic.		
Eryngium campestre L. (*Umbelliferae*)	Vetrovalj	*Eryngii radix*	Diuretic.	PDR	
Eupatorium cannabinum L. (*Compositae*)	Konopljuša	*Eupatorii cannabini herba*	Bitter tonic, immunostimulant, cytotoxic.	PDR	
Fagus sylvatica L. subsp. *moesiaca* (K. Mal'y) Szafer (*Fagaceae*)	Bukva	*Fagi pyroleum*	Antiseptic, anti-Dandruff, antiscabetic.		
Filipendula vulgaris Moench (*Rosaceae*)	Suručica	*Filipendulae radix et herba*	Diarrhea, epilepsy, kidney stones.		
Fragaria vesca L. (*Rosaceae*)	Jagoda	*Fragariae folium*	Diuretic, laxative, diarrhea, haemorrhoids.	PDR, E-	3
Frangula alnus Miller (*Rhamnaceae*)	Krušina	*Frangulae cortex*	Purgative, constipation.	Ph Eur, BP, PDR, E+, WHO	3
Fraxinus excelsior L. (*Oleaceae*)	Beli jasen	*Fraxini folium, F. cortex, F. semen*	Astrigent, diuretic, diaphoretic, antirheumatic.	Ph Eur, BP, PDR, E-	
Fraxinus ornus L. (*Oleaceae*)	Crni jasen	*Manna*	Laxative, diuretic.	Ph Eur, PDR, E+	
Galega officinalis L. (*Leguminosae*)	Ždraljevina	*Galegae officinalis herba*	Diuretic, antidiabetic.	PDR, E-	
Galeopsis speciosa Miller (*Labiatae*)	Zijevčica	*Galeopsidis herba*	Mild expectorant, astrigent.		
Galium odoratum (L.) Scop. (*Rubiaceae*)	Lazarkinja	*Asperulae herba*	Antiphlogistine, mild sedative, expectorant.	PDR, E-	3
Galium verum L. (*Rubiaceae*)	Ivanjsko cveće	*Galii veri herba*	Diuretic, diaphoretic, antispasmodic, sedative. Externally, for skin ailments, wounds, ulcers and acne.	PDR	3
Genista tinctoria L. (*Leguminosae*)	Žutičica	*Genistae tinctoriae herba, G. t. flos*	Diuretic, laxative, diaphoretic.	PDR	
Gentiana cruciata L. (*Gentianaceae*)	Prostrel	*Gentianae cruciatae radix et herba*	Stomachic, cholagogue, tonic, for appetite.		3
Geranium macrorrhizum L. (*Geraniaceae*)	Zdravac	*Geranii macrorrhizi herba*	Astrigent.		3
Geranium robertianum L. (*Geraniaceae*)	Živa trava	*Geranii robertiani herba*	Astrigent, diarrhea, gastritis, dysentery.	PDR	3
Geum urbanum L. (*Rosaceae*)	Zečja stopa	*Gei urbani rhizoma, G. u. herba*	Astrigent, hemostatic, roborantium, diarrhea.	PDR	
Glechoma hederacea L. (*Labiatae*)	Dobričica	*Glechomae herba*	Stomachic, mild expectorant, astrigent, cholagogue, diuretic.	PDR	3
Glechoma hirsuta Waldst. & Kit. (*Labiatae*)	Dobričica dlakava	*Glechomae hirsutae herba*	Same as *G. hederacea.*		
Gymnadenia conopsea (L.) R. Br. (*Orchidaceae*)	Vranjak	*Salep tuber*	Same as *O. simia.*		1, 3
Hedera helix L. (*Araliaceae*)	Bršljan	*Hederae folium*	Expectorant, antispasmodic, antimicrobial, anti-cellulite.	Ph Eur, BP, PDR, E+	3
Helianthus tuberosus L. (*Compositae*)	Čičoka	*Helianthii tuberosi radix*	Antidiabetic.		
Humulus lupulus L. (*Cannabaceae*)	Hmelj	*Lupuli strobuli*	Sedative, hypnotic.	Ph Eur, BP, PDR, WHO	
Hyoscyamus niger L. (*Solanaceae*)	Bunika	*Hyoscyami folium*	Spasms of the gastrintestinal tract.	PDR, E+	
Hypericum perforatum L. (*Clusiaceae*)	Kantarion	*Hyperici herba*	Antidepressant, astrigent, antiseptic, antiphlogistine, antiviral, gastritis, burns, wounds, cuts.	Ph Eur, BP, PDR, E+, WHO	3
Hyssopus officinalis L. (*Labiatae*)	Miloduh	*Hyssopi herba*	Antimicrobial, antiviral, carminative, mild antispasmodic, chronic bronchitis, asthma.	PDR, E-	3

Isatis tinctoria L. (*Cruciferae*)	Sač	*Isatis tinctoriae folium*	Vitamin C deficiency.		
Juglans regia L. (*Juglandaceae*)	Orah	*Juglanidis folium*	Astrigent, diarrhea.	PDR, E+	
Lamium purpureum L. (*Labiatae*)	Mrtva kopriva	*Lamii purpuree herba, L. p. flos*	Mild astrigent, antihemorrhagic, mucilaginous.		
Ligustrum vulgare L. (*Oleaceae*)	Kalina	*Ligustri folium, L. fructus*	Diarrhea.		
Lilium martagon L. (*Liliaceae*)	Ljiljan	*Lilii martagoni bulbus, L. m. flos*	Anti-inflammatory, antiseptic, cuts, wounds, burns, ulcers, frostbites.	PDR	
Linaria vulgaris Miller (*Scrophulariaceae*)	Lanilist	*Linariae herba*	Anti-inflammatory, diuretic, diaphoretic, hemorrhoids, ulcers.	PDR	
Lycopus europaeus L. (*Labiatae*)	Vučja noga	*Lycopi herba*	Antigonadotropic, antithyroid.	PDR, E+	
Lysimachia nummularia L. (*Primulaceae*)	Protivak	*Lysimachiae herba*	Same as *L. punctata.*	PDR	
Lysimachia punctata L. (*Primulaceae*)	Protivak jednocvetan	*Lysimachiae herba*	Styptic, diarrhea.		
Lysimachia vulgaris L. (*Primulaceae*)	Trava od metilja	*Lysimachiae herba*	Same as *L. punctata.*	PDR	
Lythrum salicaria L. (*Lythraceae*)	Potočnjak	*Salicariae herba*	Astrigent, hemostatic, diarrhea.	Ph Eur, BP, PDR	
Malva sylvestris L. (*Malvaceae*)	Crni slez	*Malvae flos, M. folium*	Mucilaginous, expectorant, antitussive, bronchitis, asthma.	Ph Eur, BP, PDR, E+	
Marrubium peregrinum L. (*Labiatae*)	Očajnica	*Marrubii peregrini herba*	Digestive, catarrh of the respiratory tract.		
Matricaria chamomilla L. (*Compositae*)	Kamilica	*Chamomillae flos*	Antiseptic, carminative, mild sedative, antiphlogistine, gastrointestinal spasms, cough, bronchitis, fever and colds, inflammation of the skin, mouth and pharynx, wounds and burns.	Ph Eur, BP, PDR, E+, WHO	
Medicago falcata (L.) Hudson (*Leguminosae*)	Žuta lucerka	*Medicago herba*	Sedative.		
Medicago lupulina (L.) Hudson (*Leguminosae*)	Dunjica	*Medicago lupulinae herba*	Hemostatic.		
Medicago sativa L. (*Leguminosae*)	Lucerka	*Medicago sativae herba*	Vitamin C deficiency.	PDR	
Melilotus albus Medicus (*Leguminosae*)	Kokotac beli	*Meliloti albi herba*	Similar as *M. officinalis.*		
Melilotus officinalis (L.) Pallas (*Leguminosae*)	Ždraljevina	*Meliloti herba*	Edema, thrombophlebitis.	Ph Eur, BP, PDR, E+	3
Melissa officinalis L. (*Labiatae*)	Matičnjak	*Melissae folium*	Sedative, antispasmodic, carminative, antibacterial, virostatic, migraine.	Ph Eur, BP, PDR, E+, WHO	3
Melittis melissophyllum L. (*Labiatae*)	Matočika	*Melittis herba*	Insomnia.		
Mentha arvensis L. (*Labiatae*)	Vodena metvica	*Menthae arvensis aetheroleum*	Carminative, cholagogue, antibacterial, secretolytic, cooling.	Ph Eur, BP, PDR, E+	
Mentha longifolia (L.) Hudson (*Labiatae*)	Konjski bosiljak	*Menthae longifoliae folium*	Carminative, antispasmodic.	PDR	
Mentha pulegium L. (*Labiatae*)	Barska nana	*Pulegii herba*	Carminative, antispasmodic, diaphoretic, antiseptic, sedative. Only in folk medicine.	PDR	
Nepeta nuda L. (*Labiatae*)	Macina trava glatka	*Nepetae nudae herba*	Carminative, colics, nervous disorders, migraine.		
Nigella arvensis L. (*Ranunculaceae*)	Mačkovi brkovi	*Nigellae (arvensis) semen*	Diuretic, carminative, anthelmintic, choleretic.		
Ononis spinosa L. subsp. *hircina* (Jacq.) Gams (*Leguminosae*)	Zečiji trn	*Ononidis radix*	Diuretic, kidney and bladder stone, rheumatism, gout.	Ph Eur, BP, PDR, E+	3
Orchis simia Lam. (*Orchidaceae*)	Majmunoliki kaćun	*Salep tuber*	Antitussive, mucilaginous, diarrhea, hoarseness, cough.	PDR	1, 3

Origanum vulgare L. (*Labiatae*)	Vranilova trava	*Origani herba*	Antimicrobial, cough, bronchitis, diarrhea, infections of urinary tract.	Ph Eur, PDR, E-, WHO	3
Papaver dubium L. (*Papaveraceae*)	Turčinak beli	*Papaveris dubii flos*	Mild antitussive, sedative.		
Pastinaca sativa L. subsp. u*rens* (Req. ex Godron) Čelak. (*Umbelliferae*)	Pastrnjak	*Pastinacae fructus*	Hypotensive, vitiligo.	PDR	
Persicaria hydropiper (L.) Delarbre (*Polygonaceae*)	Lisac	*Polygoni hydropiperis herba*	Antirheumatic, diuretic.	PDR	
Physalis alkekengi L. (*Solanaceae*)	Ljoskavac	*Alkekengi fructus*	Diuretic, kidney stones, rheumatism, gout.	PDR	
Pilosella officinarum Vaill. (*Compositae*)	Zečja loboda	*Hieracii herba*	Diarrhea, kidney ailments, pulmonary ailments.	PDR	
Pimpinella saxifraga L. (*Umbelliferae*)	Bedrinac	*Pimpinellae herba, P. radix*	Lung ailments. Externally for varicose veins (herba). Catarrhs of the upper respiratory tract (radix).	E-	
Pinus nigra Arnold (*Pinaceae*)	Crni bor	*Pini aetheroleum*	Catarrhal diseases of the upper and lower respiratory tract.	PDR, E+	
Plantago lanceolata L. (*Plantaginaceae*)	Muška bokvica	*Plantaginis lanceolatae folium (herba)*	Astrigent, antibacterial, catarrh of the respiratory tract, stomatitis, skin injuries.	Ph Eur, BP, PDR, E+	
Plantago major L. (*Plantaginaceae*)	Ženska bokvica	*Plantaginis majoris folium*	Diuretic, cystitis with hematuria, haemorrhoids, chronic bronchitis.	WHO	
Plantago media L. (*Plantaginaceae*)	Srednja bokvica	*Plantaginis mediae folium*	Respiratory disorders, digestive disorders, hemorrhoids, inflammation of the skin.		
Platanthera chlorantha (Custer) Reichenb. (*Orchidaceae*)	Vimenjak	*Salep tuber*	Same as *O. simia.*		1, 2
Polygala vulgaris L. (*Polygalaceae*)	Kija	*Polygalae herba*	Expectorant, chronic bronchitis, gastritis, enteritis, dyspepsia.		
Polygonum aviculare L. (*Polygonaceae*)	Troskot	*Polygoni avicularis herba*	Expectorant, astrigent.	Ph Eur, BP, PDR, E+, WHO	
Polypodium vulgare L. (*Polypodiaceae*)	Slatka paprat	*Polypodii rhizoma*	Expectorant, antiasthmatic, laxative.		
Populus nigra L. (*Salicaceae*)	Crna topola	*Populi gemma*	Superficial skin injuries, external haemorrhoids, frostbite and sunburn.	PDR, E+	
Potentilla reptans L. (*Rosaceae*)	Petoprsnica	*Potentillae reptans herba*	Astrigent, diarrhea, mouth and throat rinsing.	PDR	
Primula veris L. (*Primulaceae*)	Jagorčevina	*Primulae radix, P. flores cum (sine) calycibus*	Expectorant, nervous agitation, hysteria.	Ph Eur, BP, PDR, E+	3
Prunella vulgaris L. (*Labiatae*)	Crnj	*Prunellae herba*	Throat inflammations.	PDR	
Prunus mahaleb L. (*Rosaceae*)	Rašeljka	*Pruni mahalebi flos, P. m. fructus*	Similar as *P. spinosa.*		
Prunus spinosa L. (*Rosaceae*)	Trnjina	*Pruni spinosi flos*	Expectorant, diuretic, cold, constipation, mouth and throat rinsing.	PDR, E-	3
Pulmonaria officinalis L. (*Boraginaceae*)	Plućnjak	*Pulmonariae herba, P. folium*	Expectorant, mild diuretic, mucilaginous, astrigent.	PDR, E-	3
Quercus cerris (K. Maly) Czecz. (*Fagaceae*)	Cer	*Quercus cortex*	Astrigent, mild virostatic, diarrhea, inflammation of the skin and mucous membranes.	Ph Eur, BP, E+	
Quercus frainetto Ten. (*Fagaceae*)	Sladun	*Quercus cortex*	Same as *Q. cerris.*	E+	
Quercus petraea (Mattuschka) Liebl. (*Fagaceae*)	Kitnjak	*Quercus cortex*	Same as *Q. cerris.*	Ph Eur, BP, PDR, E+	
Quercus pubescens Willd. (*Fagaceae*)	Medunac	*Quercus cortex*	Same as *Q. cerris.*	Ph Eur, BP, E+	
Rorippa sylvestris (L.) Besser (*Cruciferae*)	Žutenica	*Rorippae folium et herba recens*	Diaphoretic, antidiabetic, anemia.		
Rosa canina L. (*Rosaceae*)	Šipak	*Rosae fructus*	Astrigent, tonic, mild diuretic, colds, vitamin C deficiency.	Ph Eur, BP, PDR, E-	3

Rosa gallica L. (*Rosaceae*)	Ruža mesečarka	*Rosae fructus*	Same as *R. canina.*	BP, PDR	
Rubus caesius L. (*Rosaceae*)	Kupina	*Rubi folium*	Astrigent, mild hypoglycemic, diarrhea.	PDR, E+	
Rumex acetosa L. *(Polygonaceae)*	Kiseljak veliki	*Rumicis acetosae herba*	Diuretic, astrigent, for anemia.	PDR	
Rumex conglomeratus Murray (*Polygonaceae*)	Kiseljak	*Rumici conglomerati herba*	Same as *R. acetosa.*		
Rumex patientia L. (*Polygonaceae*)	Zelje	*Lapathi hortensis radix*	Same as *R. acetosa.*		
Rumex sanguineus L. (*Polygonaceae*)	Kiseljak veliki	*Rumicis acetosae herba*	Diuretic, astrigent, for anemia.	PDR	
Ruscus aculeatus L. (*Asparagaceae*)	Veprina	*Rusci aculeati rhizoma*	Chronic venous insufficiency, hemorrhoids.	Ph Eur, BP, PDR, E+	3
Salix alba L. (*Salicaceae*)	Bela vrba	*Salicis cortex*	Antipyretic, antiphlogistic, analgetic, fever, rheumatism, headaches.	Ph Eur, BP, PDR, E+, WHO	
Salix caprea L. (*Salicaceae*)	Iva	*Salicis cortex*	Same as *S. alba.*	E+	
Salix fragilis L. (*Salicaceae*)	Krta vrba	*Salicis cortex*	Same as *S. alba.*	Ph Eur, BP, PDR, E+, WHO	
Salix purpurea L. (*Salicaceae*)	Rakita	*Salicis cortex*	Same as *S. alba.*	Ph Eur, BP, PDR, E+, WHO	
Salvia nemorosa L. (*Labiatae*)	Plavetnik	*Salviae nemorosae herba cum floribus*	Similar as *S. sclarea.*		
Salvia pratensis L. (*Labiatae*)	Divlja žalfija	*Salviae pratensis herba cum floribus*	Similar as *S. sclarea.*		
Salvia sclarea L. (*Labiatae*)	Mečje uvo	*Salviae sclareae herba cum floribus*	Polyarthritis, osteomyelitis.	Ph Eur	
Sambucus ebulus L. (*Caprifoliaceae*)	Apta	*Ebuli radix, E. fructus*	Diuretic, diaphoretic, purgative, neuralgia, rheumatism, gout.	PDR	
Sambucus nigra L. (*Caprifoliaceae*)	Zova	*Sambuci flos sine stipitas*	Diaphoretic, diuretic, laxative, anti-inflammatory, colds, influenza.	Ph Eur, BP, PDR, E+, WHO	3
Sanguisorba minor Scop. (*Rosaceae*)	Dinjica	*Sanguisorbae minoris herba*	Astrigent, styptic, diarrhea, hemorrhoids.		
Saponaria officinalis L. (*Caryophyllaceae*)	Sapunjača	*Saponariae radix*	Expectorant, eczema, analgesic.	PDR, E+	
Satureja kitaibelii Wierzb. (*Labiatae*)	Rtanjski čaj	*Saturejae kitaibelii herba*	Antiseptic, digestive, diuretic.		3
Sedum maximum (L.) Hoffm. (*Crassulaceae*)	Bobovnik	*Sedi maximi folium recens*	Burns, ulcers, edema.		
Sisymbrium officinale (L.) Scop. (*Cruciferae*)	Osak	*Sisymbrii folium recens, S. herba recens*	Expectorant, hoarseness.	PDR	
Solidago virgaurea L. (*Compositae*)	Zlatnica	*Virgaureae herba*	Diuretic, antiphlogistine, antispasmodic.	Ph Eur, BP, PDR, E+	3
Sorbus domestica L. (*Rosaceae*)	Oskoruša	*Sorbi domesticae fructus*	Tonic, diarrhea.	PDR	
Sorbus torminalis (L.) Crantz (*Rosaceae*)	Brekinja	*Sorbi (torminalis) fructus*	Similar as *S.domestica.*	PDR	
Stachys officinalis (L.) Trevisan (*Labiatae*)	Ranilist	*Betonicae herba*	Astrigent, sedative, diarrhea.	PDR	
Stachys palustris L. (*Labiatae*)	Čistac barski crveni	*Stachydis palustris herba*	Similar as *S. sylvatica.*	PDR	
Stachys recta L. (*Labiatae*)	Čistac	*Stachydis rectae herba*	Similar as *S. sylvatica.*		
Stachys sylvatica L. (*Labiatae*)	Čistac crveni šumski	*Stachydis sylvaticae herba*	Astrigent, diarrhea.	PDR	
Symphytum officinale L. (*Boraginaceae*)	Gavez	*Symphyti radix*	Fractures, purulent wounds, astrigent, cell proliferative.	PDR, E+	3
Taraxacum officinale Weber (*Compositae*)	Maslačak	*Taraxaci folium, T. radix*	Choleretic, diuretic, laxative, cholagogue.	PDR, E+, WHO	
Teucrium chamaedrys L. (*Labiatae*)	Podubica	*Chamaedrys herba*	Spasm, diarrhea, liver, gall and kidney ailments, hemorrhoids.	PDR	3

Teucrium montanum L. (*Labiatae*)	Trava iva	*Teucrii montani herba*	Stomachic, cholagogue, tonic.		3
Thymus odoratissimus Mill. (*Labiatae*)	Majkina dušica	*Serpylli herba*	Expectorant, antiseptic, stomachic, carminative, bronchospasm, catarrh of the respiratory tract.	Ph Eur, BP, PDR, E+	
Thymus praecox Opiz subsp. *jankae* (Čelak) Jalas (*Labiatae*)	Majkina dušica	*Serpylli herba*	Expectorant, antiseptic, stomachic, carminative, bronchospasm, catarrh of the respiratory tract.	Ph Eur, BP, PDR, E+	
Thymus pulegioides L. subsp. pannonicus (All.) Kerguélen (*Labiatae*)	Majkina dušica	*Serpylli herba*	Expectorant, antiseptic, stomachic, carminative, bronchospasm, catarrh of the respiratory tract.	Ph Eur, BP, PDR, E+	
Thymus pulegioides L. subsp. p*ulegioides* (*Labiatae*)	Majkina dušica	*Serpylli herba*	Expectorant, antiseptic, stomachic, carminative, bronchospasm, catarrh of the respiratory tract.	Ph Eur, BP, PDR, E+	
Tussilago farfara L. (*Compositae*)	Podbel	*Farfarae folium, F. flos*	Expectorant, antitussive, asthma, bronchitis, laryngitis.	PDR, E+ (folium), E- (flos)	3
Ulmus glabra Hudson (*Ulmaceae*)	Brest	*Ulmi cortex (mundatus)*	Astrigent, burns, chilblains, ulcers.		
Urtica dioica L. (*Urticaceae*)	Kopriva	*Urticae folium, U. radix, U. semen*	Antihemorrhagic, hypoglycemic, diuretic, roborantium, anemia, BPH.	Ph Eur, BP, PDR, E+, WHO (radix)	
Valeriana officinalis L. (*Valerianaceae*)	Odoljen	*Valerianae radix (rhizoma)*	Sedative, hypnotic, antispasmodic, carminative, mild hypotensive, migraines.	Ph Eur, BP, PDR, E+, WHO	
Veratrum nigrum L. (*Melanthiaceae*)	Crna čemerika	*Veratri nigri rhizoma*	Antihypertensive, diarrhea, neuralgia, rheumatism, gout.		3
Verbascum phlomoides L. (*Scrophulariaceae*)	Divizma krupnocvetna	*Verbasci flos*	Expectorant, diuretic, bronchitis, cold, wound healing.	Ph Eur, BP, PDR, E+	
Verbena officinalis L. (*Verbenaceae*)	Vrbena	*Verbenae herba*	Diuretic, astrigent, cholagogue, expectorant.	BP, PDR, E-	
Veronica beccabunga L. (*Scrophulariaceae*)	Razgon	*Beccabungae herba*	Edema.	PDR	
Veronica chamaedrys L. (*Scrophulariaceae*)	Zmijina trava	*Veronicae chamaedrys herba*	Bronchitis, asthma, gastrointestinal aliments, rheumatism, arthritis.		
Veronica incana L. (*Scrophulariaceae*)	Divlja lafendija	*Veronicae incanae herba*	Similar as *V. chamaedrys.*		
Viburnum lantana L. (*Caprifoliaceae*)	Udika	*Viburni lantanae folium, V. l. fructus*	Diarrhea.		
Vinca herbacea Waldst. & Kit. (*Apocynaceae*)	Zimzelen plavičasti	*Vincae herbaceae folium*	Sedative, antihypertensive, cerebral circulatory disorders.		2
Viola odorata L. (*Violaceae*)	Ljubičica	*Violae odoratae radix (rhizoma), V. o. flos*	Expectorant, emetic, bronchitis.	PDR	3

Presence in pharmacopoeias and other relevant literature: Ph Eur - European Pharmacopoeia 5 (2005), PDR - PDR for Herbal Medicines, second edition (2000), BP - British Pharmacopoeia 2009 (2008), E+ - Commision E Positive (Approved) Monographs, E- - Commission E Negative (Unapproved) Monographs, WHO - WHO monographs on selected medicinal plants (Vols. 1, 2, 3, 4) & commonly used in the Newly Independent States (NIS).
Conservation aspect: 1 - CITES, 2 - The Ordinance on strictly protected wild species of plants, animals and fungi, 3 - The Ordinance on protected wild species of plants, animals and fungi.

Discussion

The gorges and canyons of Balkan Peninsula are characterized by pronounced floristic diversity and diverse habitat types, enabling survival of plants with different ecological demands. Numerous botanical studies since mid-20th century have significantly contributed to knowledge on flora and vegetation of many gorges in Bosnia-Herzegovina, Montenegro [32] and Macedonia, as well as within the territory of Serbia [33], including the gorge of Svrljiški Timok.

According to studies by Bogosavljević et al. [34], the vascular flora of Svrljiški Timok gorge includes more than 689 taxa at species and subspecies level. The group of medicinal and aromatic plants is also important with 27.6% of total flora, indicating the significance of this

region. In comparison with the medicinal flora of Serbia there is a significant overlap in percentage ratio of main plant classes, with the most prominent taxa of dicots (88.8%), monocots (6.6%), gymnosperms (3%) and ferns (1.6%). The greatest significance is definitively that of *Labiatae* and *Compositae*, with 20 or more species each, higher than the average number of taxa per family (14) in this plant group. The combined participation of these families is 51%, showing the greatest contribution to the total number of medicinal species in flora of the study area. These two groups were also richest in species within the overall flora of Svrljiški Timok gorge [34]. The greatest diversity is shown by family *Labiatae*, with 32 species and 20 genera, which is therefore even richer than family *Compositae* which is richer in the overall flora of the gorge.

From the aspect of taxonomic richness, the medicinal flora of Svrljiški Timok gorge is also closely matching the structure of medicinal flora in Serbia as a whole (*Labiatae* 16.2%, *Compositae* 12%, *Rosaceae* 9%, *Umbelliferae* 6.4%, *Scrophulariaceae* and *Polygonaceae* 4% each, *Leguminosae* 3.6%, *Cruciferae* 3.2%). However, regarding the richest genera in overall flora of Serbia (*Achillea*, *Rumex*, *Artemisia*, *Teucrium*, *Thymus*, *Mentha*, *Veronica*) there are some pronounced discrepancies both in qualitative and quantitative sense. Discrepancies in the taxonomic spectrum are explained by increased presence of thermophilous habitat types, including rocks and stony ground, and by representation of xerophilous vegetation types in the gorge when compared to Serbia as a whole. Such conditions are primarily suitable for an increased presence of Pontian and Mediterranean-submediterranean plants in the total flora of the study area, influencing its medicinal flora.

Almost half of the total number of medicinal plant species in the medicinal flora of Serbia was recorded in the gorge of Svrljiški Timok, indicating the importance of study area from the aspect of biodiversity and a special place among the other areas of Eastern Serbia. The slopes of Sićevačka Klisura gorge hosted 4% [35], Rtanj Mt. 25% [36, 37], and Južni Kučaj and Juhor 39% [11] of the total number of medicinal plants growing in Serbia. It must also be stressed that comparison was based on literature data and that additional research in these regions is necessary and would probably change the above analysis.

Comparison of medicinal species listed in Table 1 with the lists in modern pharmacopeias [23, 24] and other literature data on this topic [25–31] indicates overlap in 124 cases. Out of the total number of species included in Table 1, 44 species (35%) are used in preparing herbal substances recorded in the 5th European pharmacopeia. The same number is prescribed by the British pharmacopeia with overlap in 93% of cited species (each includes a set of 41 species). Commission E, an expert group formed with the goal of estimating efficiency and effectiveness of plant materials and their phyto-preparations, traditionally publishes its results in form of monographs with positive or negative scores. The positive grade (E+), indicating that a certain herbal substance was studied and shown to have consistent effect, was assigned to substances derived from 54 species of the study area. The negative grade (E-), indicating risk in using such substance and insufficient knowledge of its effects, was assigned to 20 of the studied species. It is interesting to note that the second group includes many popular medicinal plants widely used in folk medicine in Serbia, such as *Hyssopus officinalis*, *Origanum vulgare*, *Pulmonaria officinalis*, *Tussilago farfara* etc. The World Health Organization (WHO) has published several monographs on medicinal plants, with the goal of harmonizing the use of herbal-based traditional medicines and preparations throughout the world, as well as monographs for specific parts of the world and even for separate countries. The first few editions include 21 taxa from the study area. In all, there, there are 16 taxa included in all of the cited literature sources, including some of the most popular medicinal plants such as *Achillea millefolium*, *Althaea officinalis*, *Hypericum perforatum*, *Matricaria chamomilla*, *Melissa officinalis* and *Urtica dioica*. It must be stressed that some plant species from this area (*Gentiana cruciata*, *Satureja kitaibelii* and *Teucrium montanum*) are very popular in folk medicine but not included in any of the cited sources in lists of medicinal plants.

Medicinal plants are among the most important natural resources and therefore their conservation has general ecological and manifold economic importance. Implementation of numerous regulations and laws in form of international and national conventions is introducing order into conservation of plant species, with the goal of preserving the natural resources. The Ordinance on protected and strictly protected wild species of plants, animals and fungi (Official Gazette of the Republic Serbia No. 36/09) lists several medicinal plants growing in the area of Svrljiški Timok gorge. *Adonis vernalis*, *Agrimonia eupatoria* subsp. *grandis*, *Platanthera chlorantha*, *Vinca herbacea*, as relatively rare in the wild, were placed under protection of first degree, and collecting, direct destruction or any other activities that may pose a threat to these species are forbidden, or their collecting and harvesting is allowed only in certain amounts. Some of he most important species are additionally protected by international CITES convention [38]: *Anacamptis morio*, *A. pyramidalis*, *Gymnadenia conopsea* and *Orchis simia*, referring the endangered medicinal orchid species recorded from the field.

Conclusion

Within the medicinal flora of Serbia, the flora of medicinal plants in the gorge of Svrljiški Timok stands out as rich and taxonomically diverse and distinctive. It is composed of 190 species and subspecies of vascular plants, while the families with the greatest number of medicinal representatives are *Labiatae* and *Compositae*. Out of the total number of recorded medicinal plants in this area, use of 16 plant species and their herbal substance

was recommended by most relevant pharmacopeias. On the other hand, only a relatively low percentage (25.8%) of medicinal flora in gorge of Svrljiški Timok is under legal protection. According to a number of parameters, including floral richness in medicinal plants, the gorge of Svrljiški Timok is an area important from the aspect of biodiversity conservation. Therefore it is necessary to implement the principles of nature conservation and rational use of natural resources.

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References

1. Tasić S, Šavkin-Fodulović K, Menković N, Stevanović V. Vodič kroz svet lekovitog bilja. Valjevac: Valjevo, 2004. (Serbian)
2. Sarić M. Lekovite biljke SR Srbije. SANU: Beograd, 1989. (Serbian)
3. Stevanović V. The red data book of flora of Serbia 1. Extinct and critically endangered taxa. University Faculty of Biology: Belgrade, 1999. (Serbian)
4. Pančić J. Jestastvenica za učenike Velike škole, deo prvi, U: Botanika po Šlajdenu. Državna štamparija: Beograd, 1868. (Serbian)
5. Pančić J. Jestastvenica za učenike Velike škole, deo drugi, U: Botanika po Šlajdenu. Državna štamparija: Beograd, 1873. (Serbian)
6. Petrović S. Lekovito bilje u Srbiji, Srpski arhiv za celokupno lekarstvo, odeljak drugi, knjiga XVI. Kralj.-srp. državna štamparija: Beograd, 1883. (Serbian)
7. Pelagić V. Stvarni narodni učitelj. Srpska književna zadruga: Beograd, 1893. (Serbian)
8. Tucakov J. Lečenje biljem – fitoterapija. Kultura: Beograd, 1971. (Serbian)
9. Ranđelović V, Zlatković B, Jušković M. Medicinal plants of Mt. Radan in South Serbia. Proceeding of the 8th Symposium of flora of Southeastern Serbia and neighbouring regions: Nis, 2005; pp 75–79. (Serbian)
10. Tucakov J, Mihajlov M. Medicinska flora Ovčara, Kablara i Jelice, SANU: Beograd, 1977. (Serbian)
11. Tucakov J, Mihajlov M. Farmakognozijska karta Južnog Kučaja, Juhora i Paraćinskog pomoravlja. SANU: Beograd, 1980. (Serbian)
12. Ranđelović N, Stamenković V, Jeremić Ž. Lekovite biljke Timočke krajine (fitoterapija I). Kulturno prosvetna zajednica: Zaječar, 1994. (Serbian)
13. Ranđelović N, Stamenković V, Jeremić Ž. Lekovite biljke Timočke krajine (fitoterapija II). Kulturno prosvetna zajednica: Zaječar, 1995. (Serbian)
14. Ranđelović V, Mitrović T, Jušković M. Farmakodinamska analiza lekovitih biljaka Vlasinske visoravni, Zbornik radova VII Simpozijuma o flori jugoistočne Srbije i susednih regiona: Dimitrovgrad, 2002; pp 111–116. (Serbian)
15. Marković M, Matović M, Pavlović D, et al. Resources of medicinal plants and herbs collector's calendar of Pirot county (Serbia). Biologica Nyssana 2010; 1: 9–21.
16. Mišić V. Šumska vegetacija klisura i kanjona istočne Srbije. Institut za biološka istraživanja "Siniša Stanković": Beograd, 1981. (Serbian)
17. Rakičević T. Klimatske karakteristike Istočne Srbije. Zbornik radova geografskog instituta "Jovan Cvijić": Beograd, 1976; pp 41–62. (Serbian)
18. THE EURO+MED PLANTBASE - the information resource for Euro-Mediterranean plant diversity. 2014. http://ww2.bgbm.org/EuroPlusMed/
19. Jordanov D (ed). Flora Reipublicae Popularis Bulgaricae, vols. 1–7. In: Aedibus Academiae Scientiarum Bulgaricae, Serdicae; 1936–1979. (Bulgarian)
20. Josifović M (ed). Flore de la Republique Socialiste de Serbie, vol. 1–9. Acaddemie Serbe des Sciences et des Arts: Belgrade, 1970–1977. (Serbian)
21. Guideline on specifications: test procedures and acceptance criteria for herbal substances, herbal preparations and herbal medicinal products/traditional herbal medicinal products. European Medicines Agency: London, 2011. http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/09/WC500113210.pdf
22. Jančić R, Stošić D, Mimica-Dukić M, Lakušić B. Aromatične biljke Srbije. NIP Dečje Novine: Beograd-Gornji Milanovac, 1995. (Serbian)
23. European pharmacopoeia, 5th edn. European Directorate for the Quality of Medicines and Healthcare: Strasbourg, 2005.
24. Vallender M (ed). British Pharmacopoeia. The Stationery Office: London, 2008.
25. Fleming T (ed). PDR for herbal medicines, 2nd edn. Medical Economics Co: Montvale NJ, 2000.
26. WHO monographs on selected medicinal plants, vol. 1. Geneva, 1999. http://apps.who.int/medicinedocs/pdf/s2200e/s2200e.pdf
27. WHO monographs on selected medicinal plants, vol. 2. Geneva, 2004. http://apps.who.int/medicinedocs/pdf/s4927e/s4927e.pdf
28. WHO monographs on selected medicinal plants, vol. 3. Geneva, 2007. http://apps.who.int/medicinedocs/en/m/abstract/Js14213e/
29. WHO monographs on selected medicinal plants, vol. 4. Geneva, 2009. http://apps.who.int/medicinedocs/en/m/abstract/Js16713e/
30. WHO monographs on selected medicinal plants commonly used in the Newly Independent States (NIS). Geneva, 2010. http://apps.who.int/medicinedocs/en/d/Js17534en/
31. Blumenthal M (ed). The complete German Commission E monographs. American Botanical Council: Austin, 1999.
32. Bulić Z, Lakušić D, Stevanović V. Comparative analyses of the vascular floras of the Morača and Cijevna canyons (Montenegro). Arch Biol Sci 2008; 60:485–492.
33. Zlatković B, Nikolić Lj, Ranđelović V, Ranđelović N, Stevanović V. Comparative analyses of the vascular floras of two gorges of Pčinja river in Serbia and Macedonia. Arch Biol Sci 2011; 63: 1157–1166.
34. Bogosavljević S, Zlatković B, Ranđelović V. Flora of the Svrljiški Timok Gorge in Eastern Serbia. Proceeding of the 9th Symposium on flora of Southeastern Serbia and neighbouring regions: Niš, 2007; pp 41–54. (Serbian)
35. Tucakov J. Biološki i farmakomedicinski značaj izvesnog mirisnog lekovitog bilja na južnim padinama Sićevske klisure. Glasnik muzeja šumarstva i lova: Beograd, 1962; pp 75–100. (Serbian)
36. Milojević B. The use of plants in the folk life in the Region of Rtanj in the east Serbia. Lekovite sirovine 1988; 7:89–101. (Serbian)
37. Zlatković B, Bogosavljević S, Radivojević A, Pavlović M. Traditional use of the native medicinal plant resource of Mt. Rtanj (Eastern Serbia): ethnobotanical evaluation and comparison. J Ethnopharmacol 2014; 151:704–713.
38. Convention on international trade in endangered species of wild fauna and flora. Washington, 1973. http://www.cites.org/sites/default/files/eng/disc/E-Text.pdf

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PHENOLIC COMPOUNDS CONTENT AND RADICAL SCAVENGING CAPACITY OF WHEAT-LENTIL DOUGH

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Abstract. *The lentil (Lens culinarisL.) is a legume plant, one of the oldest known food crops and medicinal plants. The health benefits of lentil are well known: its consumption reduces the risks of cardiovascular diseases and some cancers. It has a low glycemic food index and is important in the dietary treatment of diabetes mellitus.Unfortunately, its consumption in many countries is low. Since bread is a daily consumed food this can be improved by adding the lentil in wheat flour. In this paper the content and DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging capacity of phenolic compounds from wheat dough and dough obtained by wheat flour supplemented with 40% of lentil flour were examined and compared. The dough with lentil flour had higher content of phenolic compounds than the dough with wheat flour only (2144.7 and 1592.5 μg of chlorogenic acid/g, respectively) and achieved higher DPPH scavenging capacity (SC_{50} value was 21.2 and 56.3 mg/mL, respectively). Results showed that, after baking, the dough retained the same value of DPPH scavenging capacity, while baked wheat-lentil dough had near three times higher antioxidant activity than baked wheat dough. These investigations indicate that the lentil flour is useful food ingredient for improving the antioxidative potential of wheat flour*.

Key words: *Lentil, dough, phenolic compound, scavenging capacity*

Introduction

Plant phenolic compounds are secondary plant metabolites synthesized by plants during their normal development or in response to stress conditions such as infection, wounding and UV radiation [1, 2]. They are a highly diversified group of compounds including the simple phenolics, phenolic acids, coumarins, flavonoids, hydrolysable and condensed tannins, lignans and lignins [3]. Phenolic compounds have free radical scavenging abilities, anti-mutagenic and anti-carcinogenic activities and the ability to reduce the risk of cardiovascular and carcinogenic diseases [4]. Their content in plants depends on many factors such as cultivar and stage of ripening [5, 6] and antioxidant activity depends on phenological stage [7].

The lentil (*Lens culinaris* L.) is a legume plant, one of the oldest known food crops as it has been cultivated for more than 8,500 years ago. Legumes are well known as "the poor man's meat", widely available and inexpensive, but they are not fully exploited [8]. Legumes are important crops due to their nutritional quality. It is an excellent and inexpensive source of protein, amino acids such as L-lysine and L-arginine, complex carbohydrates, fibre and minerals [9, 10]. The health benefits of lentils are also well known: its consumption reduces the risks of cardiovascular diseases and even some cancers. They have been identified as low glycemic index foods [11] and are important in dietary treatment of diabetes mellitus as they increase satiety and facilitate the control of food intake. In the *Caenorhabditis elegans* model system, legumes reduced intestinal fat [12]. Due to these lentil abilities, adding lentil flour to wheat flour could show potential to formulate functional foods.

Unfortunately, its consumption in many western countries is low. Since bread is daily consumed food in these regions, this can be improved by adding the legumes to bread. The legumes in food products in relation to currently used breads contribute to higher content of protein, minerals, fat and fiber, change cake volume [13] and lower the content of gluten and carbohydrate [14].

In available literature there is data about the content of the phenolic compounds from lentil and wheat flour and their antioxidant activity. However, they are not determined by the same procedure and equipment, and could not be used for comparison. The purpose of this paper is to determine and compare such data, and investigate the effects of the replacement of wheat flour by the lentil flour on phenolic compounds content and radical scavenging capacity. This is useful for an evaluation of the potential of lentil flour to improve the antioxidative potential of wheat flour, and in this way

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formulated functional foods. In this paper the phenolic content and the radical scavenging capacity of wheat (WF) and lentil flour (LF), wheat-lentil flour mixture in ratio of 60:40 (w/w) (WLM), wheat dough (WD) and dough obtained from WLM mixture (WLD), as well as baked wheat dough (WB) and baked wheat-lentil dough (WLB), were examined and compared.

Material and Methods

Flours and dough

Lentil flour was obtained by milling lentil seeds originating from Canada, grown in 2012 and sieving through a 0.30 mm sieve. The used flour was analysed for moisture, protein and ash content. The moisture content was determined by Scaltec SMO 01 (Scaltec instruments, Germany) instruments: 5 g of flour was put in the disk plate analyzer, dried at 110 ^{0}C to a constant weight, and the moisture content was read out on the display. Protein content was determined by the Kjeldahl method (Nx5.95) and the ash content by staking of sample at 800^{o}C during 5 h [15]. The wheat flour type 500 ("Padež", Bunibrod, Serbia) from the crop of 2012, was bought in a local store in Leskovac, Serbia, and the same analyses of the lentil flour were performed with wheat flour. The mixture wheat-lentil flour (WLM) was obtained by mixing the wheat and the lentil flour in ratio of 60:40 (w/w).

The dough from the wheat flour only and the wheat-lentil flour mixture, were obtained by mixing using farinograph (Brabender Model 8 10 101, Duisburg, Germany) according to ISO 5530-1 test procedure. In order to obtain a sample of dough, small slices of approximately of 1 × 0.5 cm, were cut out of dough, dried at 30^{o}C during 3 h and milled and sieved through a 0.30 mm sieve.

The separate sample of dough obtained by the same mixing procedure on the farinograph was shaped into round balls, of approximately 30 cm in diameter and 2.5 cm in height and baked at 180^{o}C, for 50 minutes in the oven (Candy, FPP403/1). The baked wheat dough (WB) and wheat-lentil dough (WLB) were cooled down to room temperature and sliced to a size approximately of 25 × 1.5 cm. The slices were dried for 3 h at 30 ^{o}C, milled and sieved through a 0.30 mm riddle.

Preparation of extracts

For measurements of the phenolic compounds content in LF, WF, WLM, WD and WLD, 5 g of the flour or sample was measured and 100 mL of 80% (v/v) ethanol was added. The mixture was stirred by MR1 magnetic stirrer (IKA-Werke, Staufen, Germany) for 10 minutes at 200 min^{-1} and vacuum filtered through No. 54 Wathman filter paper (GE Healthcare, Brondby, Denmark). The solids were re-extracted with 50 mL of 80% (v/v) ethanol, the filtrates combined and made to a final volume of 150 mL. For radical scavenging capacity (SC) measurements, 140 mL of each extract was evaporated in the vacuum at 45 ^{o}C until dry, and was dissolved in 30 mL of 96% (v/v) ethanol.

Phenolic compounds content

A standard curve for five chlorogenic acid (Sigma Chemical, St. Louis, Missouri, USA) concentrations covering the range from 10 to 300 μM (C=2319×Ab-10.2) was first made for phenolic content (PCC) determination. According to method of Glories (1978) [16], 4.50 mL of 2 g/mL HCl and 0.25 mL of chlorogenic acid standard solutions was added, mixed by vortex and allowed to stand for approximately 15 min, for PCC determination, in a test tube 0.25 mL of 0.1 g/mL HCl in 95% (v/v) ethanol. Then the absorbance (A) was read at 280 nm using UV 21000 Spectrophotometer (Cole Parmer Instruments Company, Vernon Hills, Illinois, USA).For measuring PCC in flours and dough, 0.25 mL of 0.1 g/mL HCl in 95% (v/v) ethanol, 4.50 mL of 2 g/mL HCl and 0.25 mL of filtered extracts was added into test tube and further treated as standard solutions of chlorogenic acid.

Radical scavenging capacity

The radical scavenging capacity (SC) of an extract diluted by ethanol to obtain concentrations ranging from 0.3 to 8 mg/mL, was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) test [17]. The ethanol solution of DPPH radicals concentration of 0.1 mM (1 mL) was added to 2.5 mL ethanol solution of the given concentration of the investigated extract and allowed to react at room temperature for 30 min. Then the A value was measured at 518 nm on UV 21000 Spectrophotometer (Cole Parmer Instruments Company) and converted to percentage of radical SC by using the equation defined by Mensor and Menezes (2001) [18]:

$$SC = 100 - \frac{(A_{sample} - A_{blank})}{A_{control}} \times 100$$

where A_{sample} is the absorbance at 518 nm of the ethanol solution of the extract treated by the DPPH radical solution; A_{blank} is absorbance at 518 nm of the ethanol solution of the extract (1 mL of ethanol added to 2.5 mL of extract), and $A_{control}$ is absorbance at 518 nm of ethanol solution of DPPHradical (1 mL of a 0.3 mM added to 2.5 mL of ethanol). The final results are presented as SC_{50} value, calculated by using Microsoft Excel ed50plus (v1.0) software by Mario H. Vargas, InstitutoNacionale de EnfermedadesRespiratories by inputtingthe data of SC and extract concentrations in appropriate columns and using the function "Interpolate" (www.sciencegateway.org/protocols/cellbio/drug/hcic50.htm). The value of SC_{50} represents the concentration of dry residue of studied extracts that causes a decrease in the initial DPPH concentration by 50%.

Statistical analysis

Statistical version 5.0 Software (StatSoft, Tulsa, Oklahoma, USA) was used to perform the statistical analysis: the mean, standard deviations and statistical dependence. The mean and standard deviations were obtained by using Descriptive Statistics, marking the Median & Quartiles and Confirm Limits for Means. Where appropriate, the

statistical dependence was tested by Excel 2003 and ANOVA Single factor test. Differences with $p<0.05$ were considered to be statistically significant.

Results

The moisture, protein and ash content in the tested samples is shown in Table 1. The investigated characteristics of obtained extracts from lentil and wheat flour and dough, extract yield (EY), phenolic compounds content and SC_{50} value, are also shown in Table 1. Values are the means and standard deviation (N=3) obtained by descriptive statistics and the same letters in superscript within the same column indicate significant differences ($p > 0.05$) obtained by ANOVA test.

The results of the dependence of the scavenging capacity on the concentrations of the polyphenols in the extract obtained from the investigated flours, wheat-lentil flour mixture (60:40 w/w), their dough obtained after mixing and corresponding baked dough are presented in Figure 1.

Discussion

The results presented in Table 1 show there are significant differences between flours in protein and ash content and the replacement of wheat by lentil flour increases the contents of these components, in dough as well as in final food products. In WLD, the protein content was 1.5 and ash content 3.3 times higher than in WD.

The extract yield (EY) of LF was higher than the EY of WF and the EY of the dough extract was higher than the EY of the flour extract from which they are made. The EY was 9.1 g/100 g for the extract obtained from WD and 6.6 g/100 g from WF. The EY of the extract of WLD was 12.1 and it was also two times higher than the EY of WLM, where it was 7.4 g/100g.

Han and Baik [19] published that the phenolic compounds content (PCC) in lentil (after extraction by 30% dimethylformamide and determination by using 4-aminoantipyrine and ferric cyanide and measuring absorbance at 505 nm) was ~12 mg/g expressed in galic acid equivalent. On the other hand, the PCC in lentil after extraction with acetone/water/acetic acid (70:29.5:0.5,

Table 1. The characteristics, phenolic compounds content and radical scavenging capacity of extracts obtained from lentil and wheat flour and dough

Sample /Parameter	Moisture (g/100 g)	Protein content (g/100 g)	Ash content (g/100 g)	EY (g/100 g)	PCC (μg/g)	SC_{50} (mg/mL)
LF	10.7 ± 0.6	21.9 ± 1.6^{a}	3.02 ± 0.6^{a}	8.1 ± 0.6	993.7 ± 32^{a}	2.2 ± 0.4^{a}
WF	13.1 ± 0.8	9.8 ± 0.8^{a}	0.48 ± 0.6^{a}	6.6 ± 0.8	789.6 ± 23^{a,d}	13.8 ± 0.4^{a,d}
WLM	12.8 ± 0.8	14.6 ± 1.1^{a}	1.49 ± 0.5^{a}	7.4 ± 0.9	878.9 ± 36^{a,e}	6.8 ± 0.3^{a,e}
WD	11.6 ± 0.9	9.2 ± 0.9^{b}	0.42 ± 0.6^{b}	9.1 ± 0.8	1592.5 ± 52^{b,d}	56.3 ± 0.8^{b,d}
WLD	12.3 ± 0.9	13.9 ± 1.8^{b}	1.41 ± 0.6^{b}	12.1 ± 0.9	2144.7 ± 71^{b,e}	21.2 ± 0.6^{b,e}
WB	11.8 ± 0.8	9.4 ± 0.8^{c}	0.43 ± 0.7^{c}	8.9 ± 1.1	1198 ± 64^{c}	61.4 ± 1.1^{c}
WLB	12.1 ± 1.1	14.1 ± 1.4^{c}	1.42 ± 0.4^{c}	10.9 ± 1.2	1897 ± 83^{c}	24.2 ± 0.4^{c}

Values are the means followed by standard deviation (N=3)
The same letters in superscript within the same column indicate significant differences ($p > 0.05$).

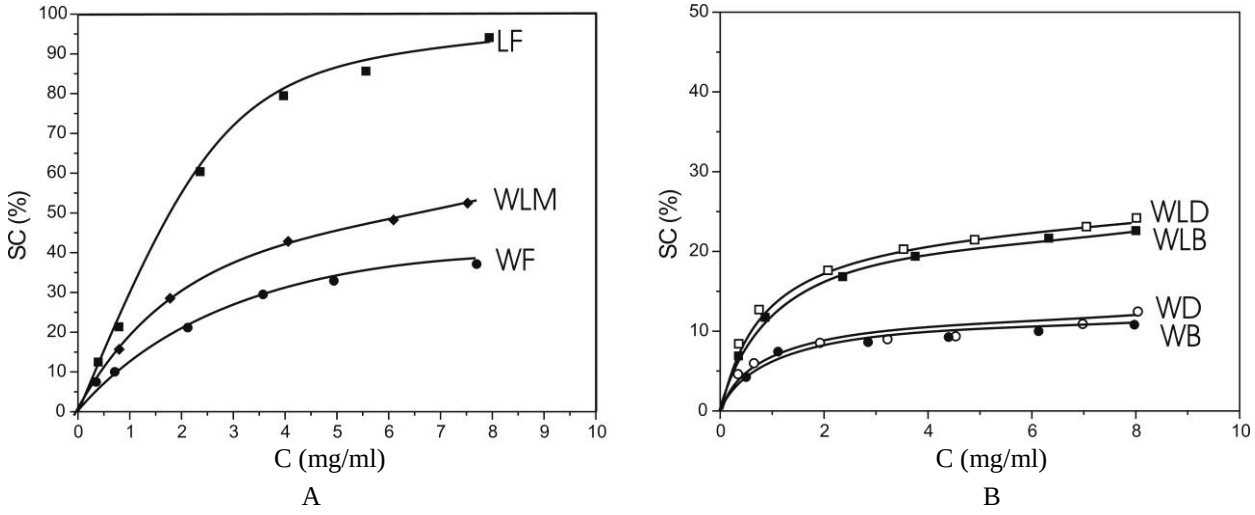

Fig. 1. The dependence of the scavenging capacity on the concentrations of polyphenols in extract obtained from the lentil flour (LF), wheat flour (WF), wheat-lentil flour mixture (60:40 w/w) (WLM) – A, and wheat dough (WD), wheat-lentil dough (WLD), baked wheat dough (WB), baked wheat-lentil dough (WLB) – B

v/v/v), determination with Follin-Ciocalteu assay, measuring absorbance at 765 nm, was 70.0 mg/g, expressed in gallic acid equivalent [20]. For wheat flour there is also an abundance of data where PCC was in the 254–499 μmol gallic acid equivalent/100g of wheat range, depending on the varieties [21] (obtained after extraction by 80% chilled acetone and by Follin-Ciocalteu reagent, measuring absorbance at 760 nm). Other studies have shown that the PCC was in the 119–201 μmol gallic acid equivalents/100g of wheat range, also depending on the varieties (obtained after extraction by 80% chilled ethanol and by Follin-Ciocalteu reagent, measuring absorbance at 760 nm) [22]. It is evident that making conclusions and comparisons based only on the presented literature data is not valid. The available literature does not provide data about the PCC and radical scavenging capacity in those doughs after processing, such as mixing and baking. These are the reasons why, in this paper, we presented and compared the results of PCC and radical scavenging capacity in lentil and wheat flour and their doughs.

The PCC in lentil flour that we have obtained was of 993.7 μg of chlorogenic acid/g and it was higher than in wheat flour (789.6). The PCC in dough was higher than in corresponding flour: WD contained 1592.5 μg/g while WLD had 2144.7 μg/g and it is 2.5 times higher than in wheat-lentil flour mixture, where it was 878.9 μg/g. Based only on these results, it is difficult to explain how the PCC appeared to be higher in a sample of dough than in corresponding samples of flour. These results could indicate that during the dough mixing process, when water was added, the reactions of hydration of phenolic compounds probably occurred. Also, phenolic compounds exist in their hydrate state and this probably increases the extractability of the phenolic compounds [23] and causes a higher value of EY and PCC in dough samples. Comparison of PCC in WLD and WD showed that value of PCC was considerably higher in the dough obtained from the mixture where wheat flour was replaced by lentil flour.

Furthermore, higher PCC in the lentil flour than in the wheat flour also caused a higher DPPH radical scavenging capacity of the extracts. The investigations showed the DPPH scavenging capacity depended on the extract concentration and it increased when the extract concentration increased. In extracts where the dry residue concentration was 8.0 mg/mL, the extract obtained from the lentil flour had a SC of 93.2%, while the extract from the wheat flour had a SC of only 34.9% (Figure 1A).

The extract obtained from WD and WLD had considerably lower SC than extracts from WF and WLM, respectively. The mixing of dough reduced DPPH scavenging capacity of WLD by approximately 25%, compared to the scavenging capacity of WLM. The reason for this might be the oxidation or hydration reactions of phenolic compounds which can occur during mixing. It is known that the processing of cereals and legumes, such as germination, may increase the level of phenolic compounds in foods when enzymatic reactions in seeds occur [24, 25].

Obtained SC_{50} values (Table 1) expressed as μg of chlorogenic acid per ml of extract were lower than the SC_{50} value obtained for ascorbic acid (9.8 μg/mL). Lower SC_{50} value indicates higher scavenging capacity which is in accordance with the results of SC. As WLD had higher SC and lower SC_{50} value than WD (Figure 1B), it was evident that the replacement of 40% of wheat flour by lentil flour improved antioxidant activity of dough, thus offering better health benefits.

Results obtained with baked wheat-lentil dough (WLB) were 21.8% for DPPH radical scavenging capacity and 24.2 mg/mL for SC_{50} value (Figure 1B). Based on these results, the baked dough retained the DPPH scavenging capacity which dough had had and bread from wheat-lentil flour mixture will have near three times higher antioxidant activity than bread made of wheat flour only: SC_{50} value for WB was 61.4 and for WLB, 24.2 mg/mL (Figure 1A). These results are in accordance with the results reported by Hye-Min and Bong-Kyung [26] when caffeic acid was added to dough and the recovery of caffeic acid after baking was 74–80%.

According to ANOVA test results, the lentil flour addition significantly affected the protein and ash content as well as phenolic compounds content and DPPH scavenging capacity ($p > 0.05$). Dough mixing also significantly affected the phenolic compounds content and DPPH scavenging capacity and baking had no significant effect on these parameters. The higher difference between F and F critical values (588 and 7.7, respectively) was observed for SC_{50} value of WF and WLM.

Conclusion

By replacing 40% of wheat flour with lentil flour, the obtained dough had a 1.3 times higher content of phenolic compounds and 2.7 times higher SC_{50} value than dough made of wheat flour only. By dough mixing, the DPPH scavenging capacity at the concentration of 8 mg/mL, for the extract obtained from wheat-lentil dough, was reduced by approximately 25%, compared to scavenging capacity of flour mixture from which it was made. Baked dough from wheat-lentil flour mixture had almost three times higher antioxidant activity than baked dough made from wheat flour only, so the addition of the lentil flour to the wheat flour showed potential to improve the antioxidant potential of wheat flour.

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References

1. Nicholson R, Hammerschmidt R. Phenolic compounds and their role in disease resistance. Annu Rev Phytopathol 1992; 30:369–389.
2. Lattanzio V, Lattanzio T, Cardinal A. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and incests. In: Imperato F (ed) Phytochemistry: Advances in Research. Research Signpos: Kerala, 2006; pp 3–67.
3. Shahidi F. Naczk M. Biosynthesis, classification and nomenclature of phenolics in food and nutraceuticals. In: Naczk M, Shahidi F (eds) Food phenolics: source, chemistry, effects, applications. CRC Press: Boca Ratton, 2004; pp 1–16.
4. Nakamura Y, Watanabe S, Miyake N, Kohno H, Osawa T. Dihydrochalcones: evaluation as novel radical scavenging antioxidants. J Agric Food Chem 2003; 51:3309–3312.
5. Toledo F, Arancibia-Avila P, Park YS, et al. Screening of the antioxidant and nutritional properties, phenolic contents and proteins of five duran cultivars. Int J Food Sci Nutr 2008; 59: 415–427.
6. Nikolić N, Cvetković D, Todorović Z. Characterization of content, composition and antioxidant capacity of phenolic compounds in celery roots. Ital J Food Sci 2011; 23:214–219.
7. Porres-Martínez M, González-Burgos E, Carretero E, Gómez-Serranillos P. Influence of phenological stage on chemical composition and antioxidant activity of Salvia lavandulifolia Vahl. essential oils. Ind Crop Prod 2014; 53:71–77.
8. Duranti M, Morazzoni P. Nutraceutical properties of lupin seed proteins. A great potential still waiting for full exploitation. Agro Food Ind Hi Tec 2011; 22:20–25.
9. Salunkhe K, Kadam S. Handbook of word legumes: Nutritional chemistry, processing technology and utilization. CRC Press: Florida, 1989.
10. Longnecker N, Kelly R, Huang S. The lentil lifestyle-Health benefits of lentil and their use in diets. In: Brouwer JB (ed) Proceedings of Lentil Focus. National Conference, Horsham (Victoria), 2002; pp 58–59.
11. Bornet FR, Billaux MS, Messing B. Glycaemic index concept and metabolic diseases. Int J Biol Macromol 1997; 21:207–219.
12. Finley JW, Sandlin C, Holliday DL, Keenan MJ, Prinyawiwatkul W, Zheng J. Legumes reduced intestinal fat deposition in the Caenorhabditis elegans model system. J Funct Foods 2013; 5:1487–1493.
13. Gularte MA, Gómez M, Rosell CM. Impact of legume flours on quality and in vitro digestibility of starch and protein from gluten-free cakes. Food Bioprocess Technol 2012; 5:3142–3150.
14. Mohamed AA, Rayas–Duarte P, Shogren RL, Sessa DJ. Low carbohydrates bread: Formulation, processing and sensory quality. Food Chem 2006; 99:686–692.
15. Trajković J, Baras J, Milić S, Šiler S. Analiza životnih namirnica. Tehnološko-metalurški fakultet: Beograd, 1983. (Serbian)
16. Glories Y. Recherches sur la matière colorante des vins rouges. These Doctorat d'Etat. Universitè de Bordeaux II: Bordeaux, 1978. (French)
17. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. J Chromatogr A 2004; 1054:95–111.
18. Mensor LL, Menezes FS, Leitão GG, et al. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2001; 15:127–130.
19. Han H, Baik B-K. Antioxidant activity and phenolic content of lentils (Lens culinaris), chickpeas (Cicer arietinum L.), peas (Pisum sativum L.) and soybeans (Glycine max), and their quantitative changes during processing. Int J Food Sci Tech 2008; 43:1971–1978.
20. Zou Y, Chang SK, Gu Y, Qian SY. Antioxidant activity and phenolic compositions of lentil (Lens culinaris var. Morton) extract and its fractions. J Agric Food Chem 2011; 59: 2268–2276.
21. Okarter N, Liu CS, Sorrells ME, Liu RH. Phytochemical content and antioxidant activity of six diverse varieties of whole wheat. Food Chem 2010; 119:249–257.
22. Adom KK, Sorrells ME, Liu RH. Phytochemical profiles and antioxidant activity of wheat varieties. J Agric Food Chem 2003; 51:7825–7834.
23. Hughey CA, Januszewicz R, Minardi CS, et al. Distribution of almond polyphenols in blanch water and skins as a function of blanching time and temperature. Food Chem 2012; 131:1165–1173.
24. Kwaku D. Effects of processing on antioxidant phenolics of cereal and legume grains. In: Awika JM, Piironen V, Scott B (eds) Advances in cereal science: Implications to food processing and health promotion. ACS Publication: Washington, 2011; pp 31–54.
25. Ghavidel RA, Prakash J, Davoodi MG. Assessment of enzymatic changes in some legume seeds during germination. Agro Food Ind Hi Tec 2011; 22:45–47.
26. Han HM, Koh BK. Antioxidant activity of hard wheat flour, dough and bread prepared using various processes with the addition of different phenolic acids. J Sci Food Agric 2011; 91:604–608.

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CD34 AND C-KIT IMMUNOREACTIVE CELLS IN THE HUMAN EMBRYONAL AND FETAL SMALL BOWEL

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Abstract. *Interstitial cells of Cajal (ICC) play important roles in the control of digestive motility: they generate the electrical slow-wave activity (pacemaker component) of the gut musculature and are involved in neurotransmission and stretch sensation. ICC expresses c-kit and depends on signaling via Kit receptors for development and maintenance of phenotype. The aim of the present study was to investigate if the c-kit immunoreactive (IR) cells present in the wall of the small bowel at the beginning of the fetal period are CD34 immunopositive. Human small bowel specimens were obtained from 5 embryos and 7 foetuses, 7–12 weeks of gestational age. The specimens were exposed to anti-c-kit antibodies to investigate ICC differentiation and anti-CD34 antibodies to identify presumed ICC progenitors. The differentiation of smooth muscle cells was studied with anti-desmin antibodies. At 9–10 weeks, c-kit IR cells were present in the wall of small bowel in the form of a narrow band of cells, at the level of the myenteric plexus, but they were absent in the mucosa and submucosa of the gut. At the same time, CD34 IR cells were present at the level of submucosa and mucosa, and they were not present in the outer parts of gut wall. A clear distinction between the localization of c-kit IR cells and CD34 IR cells was evident. We may conclude that c-kit IR cells present in the small bowel wall at the beginning of fetal period of development, at 9–10 weeks, do not exhibit concurrent CD34 immunoreactivity.*

Key words: *Small bowel, c-kit, CD-34, immunohistohemistry, human*

Introduction

Interstitial cells of Cajal (ICC) are a distinct and unique cell population distributed in the muscle layer of digestive tube of many vertebrates including humans [1, 2]. They are network-forming cells connected electrically with each other and with smooth muscle cells via gap junctions [3, 4]. ICC play important roles in the control of digestive motility: they generate the electrical slow-wave activity (pacemaker component) of the gut musculature [5–7] and are involved in neurotransmission [8, 9] and stretch sensation [10]. ICC express c-kit and depend on signaling via Kit receptors for development and maintenance of phenotype [11].

At the end of the embryonic period of human development, c-kit immunoreactive (c-kit IR) cells are present in the oesophagus and stomach wall in the form of a wide belt of cells around the inception of the myenteric plexus (MP) ganglia [12, 13]. In the small and large bowel, c-kit-IR cells appear later (in the small bowel at 9 weeks, and in the colon at 10–12 weeks), in the form of narrow linear rows of cells, also in the MP region [14–16].

Enteric neurons and glial cells arise from the neural crest (from "vagal" and "sacral" level) [17]. Lecoin et al. [18] were the first to show that ICC in the avian intestine do not arise from the neural crest. The study by Klüppel et al. [19] also suggested strongly that ICC and smooth muscle cells arise from common precursor cells. Smooth muscle markers, such as the heavy chain of smooth muscle myosin, are coexpressed with Kit in the developing gut [19]. However, some of the Kit-positive mesenchymal cells are destined to become smooth muscle cells, and such cells down-regulate the expression of Kit and unregulate the expression of myofilament proteins. ICC maintains their expression of Kit, even in mature animals.

Lorincz et al. [20] provided evidence for characterization of potential progenitor cells for ICC in the stomach of adult mice. They have demonstrated that these cells are positive for Kit, CD34 (an adhesion molecule), as well as CD44, insulin receptors and IGF-I receptors (IGF-IR) [20]. CD34-cells, mostly known as interstitial Cajal-like cells (ICLCs), are present in the submucosa of the entire human gastrointestinal tract [21]. Recently, telocytes, belonging to the group of ICLCs, were described as a distinctive type of cells [22]. The ICC precursor cell could possibly appear as a fibroblast-like (immature)

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ICC at the ultrastructural level, identified in W mutant animals; these cells are Kit negative, but have gap junction contact with smooth muscle cells and are also associated with Auerbach's plexus [23].

The identification of the morphology of the immature ICC, their cytological changes and their organization during differentiation might help in interpreting the significance of abnormalities in the ICC distribution, density and morphology at birth and in the early paediatric age, as well as in understanding the pathophysiology of intestinal motile disorders in neonates and young children.

The aim of the present study was to investigate if the c-kit IR cells present in the wall of the small bowel at the beginning of the foetal period are CD34 immunopositive, respectively if they represent the common precursors of ICC and smooth muscle cells, or already differentiated, mature ICC.

Material and Methods

The human material was obtained after legal abortions (0.5–1 h postmortem) and premature births due to pre-partial deaths according to the principles of the Ethical Committee of the Faculty of Medicine of the University of Niš. Both genders are represented in the sample, and no specimens had gastrointestinal disorders. Gestational ages were estimated by anatomic criteria according to the Carnegie Staging system and the crown-rump length, head circumference, and foot length. Each embryo and foetal small bowel specimen was fixed in 10% neutral formalin and paraffin-embedded. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Niš.

The study material consisted of 5 human embryos and 7 human foetuses, 7–12 weeks gestational age (7 weeks, n=2; 8 weeks, n=3; 9 weeks, n=2; 10 weeks, n=2; 12 weeks, n=3). Small foetuses (9 and 10 weeks) were processed completely, sequentially sectioned at 4 μm, and stained. Immunohistochemical analysis was performed using the detection Kit-Polymer. The sections were deparaffined in xylol and a descending series of alcohol rinses (< 1 min each), then rehydrated in distilled water. The endogenous peroxidase was blocked with 3% H_2O_2 for 10 min at room temperature. This was followed by incubation with the primary antibodies for 60 min at room temperature, rinsing in a phosphate buffered solution (0.1M PBS, pH 7.4). The primary antibodies were dissolved in Dako antibody diluent (Cat. No. S0809). The sections were incubated with streptavidin horseradish conjugate for 30 min at room temperature. The complex was visualised with DAKO Liquid DAB + Substrate/ Chromogen System (Code No. K3468) and DAKO AEC + Substrate/Chromogen System (code no. K3469; Dako). Immunostaining for CD34 was then performed as previously described. All immunolabelled sections were counterstained by Mayer's haematoxylin. Immunoreactivity was absent in negative controls in which the primary antibody was omitted. Sections were examined with an Olympus BX50 microscope and photographed with an Olympus PM-C35 camera.

The primary antibodies used, and their respective dilutions, are listed in Table 1.

Table 1. Antibodies

Antigen	Clone	Supplier	Dilution
C-kit	CD-117	Dako	1 : 300
CD34	QBEnd 10 N1632	Dako	Ready to use
Desmin	DE-R-11	Dako	1 : 100

Results

In the study, we have not considered the initial portions of the small bowel, immediately adjacent to the stomach, developing from the foregut, in which ICC differentiate in the way identical to that in the stomach.

At the end of the embryonic period of development, at weeks 7 and 8, DES immunoreactivity was faint in the cells that would form the circular muscle layer. In the same period, c-kit IR cells were absent in the wall of the midgut, part of the primitive gut which gives a rise to small bowel. CD34 IR cells were present in the inner parts of the wall of the primitive gut, which will develop in mucosa and submucosa, but not present at the level of the MP ganglia. At 9–10 weeks, DES immunoreactivity was present in all parts of the small bowel. DES immunostaining was observed as a band that encircled the gut (corresponded to the circular muscle layer), and in the form of an extremely thin band of cells located outside the MP (presumptive longitudinal muscle layer) (Fig. 1).

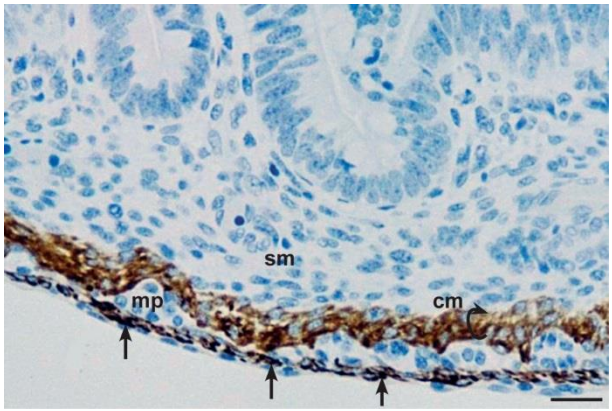

Fig. 1. Desmin immunohistochemistry (9 weeks gestational age, small bowel). DES-IR was present in the circular muscle layer and in the very thin band of cells located outside the MP, presumptive longitudinal muscle layer (*arrows*). cm, circular muscle layer; mp, myenteric plexus; sm, submucosa. Bar: 30 μm.

In this period of development, c-kit IR cells were detected in the wall of small bowel. They were present in the form of a narrow band of cells, at the level of the MP and encircled the ganglia, but neither their bodies nor their processes were present within the ganglia. C-kit IR cells were absent in the mucosa and submucosa of the gut (Fig. 2).

At the same time, CD34 IR cells were present and distributed in the identical way as in the embryonal period, at the level of submucosa and mucosa, and they were not present in the outer parts of gut wall (Fig. 3). A clear distinction between the localization of c-kit IR cells and CD34 IR cells was evident (Figs. 2 and 3).

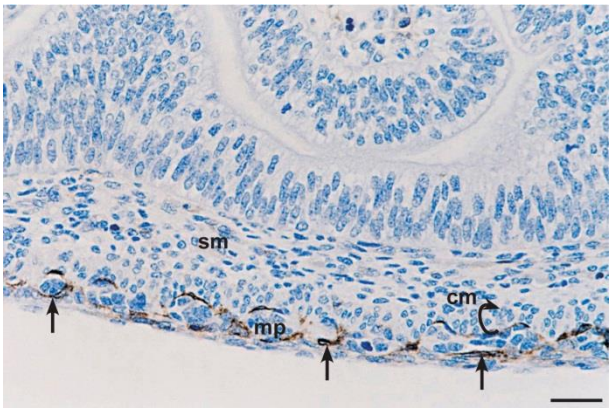

Fig. 2. C-kit immunohistochemistry **(**9 weeks gestational age, small bowel). C-kit-IR cells (*arrows*) located in the outer layers of the developing small bowel, surrounding the presumptive myenteric ganglia. cm, circular muscle layer; mp, myenteric plexus; sm, submucosa. Bar: 30 µm.

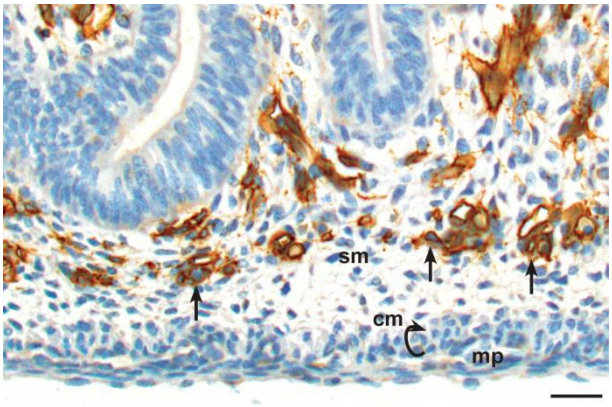

Fig. 3. CD34 immunohistochemistry (9 weeks gestational age, small bowel). CD34 IR cells (*arrows*) were present in the inner parts of the wall of the primitive gut, which will develop in mucosa and submucosa, but not present at the level of the MP ganglia. cm, circular muscle layer; mp, myenteric plexus; sm, submucosa. Bar: 30 µm.

By weeks 11–12, differences in the distribution of c-kit IR cells and CD34 IR cells which were described above, were present along the entire length of the small bowel.

Discussion

The paper described the time and mode of differentiation of muscle layers of the small bowel wall, primarily aiming to determine in a more precise way the place of appearance and mode of distribution of c-kit IR cells. Namely, at the end of embryonic period, the circular muscle layer differentiated, while the longitudinal appeared at the beginning of foetal period of development. These findings are in agreement with previous results regarding the development of neuromuscular structures in the human small bowel [24, 25].

Our results demonstrated that c-kit IR cells appeared in the small bowel wall (with the exception of its initial portion due to reasons mentioned above) not till the foetal period of development and in the myenteric plexus region, without being present in the inner parts of the wall, in the region of submucosa and mucosa of the bowel. On the other hand, CD34 IR cells were present at the end of embryonic period and the beginning of foetal period, but in both cases only in the inner parts of the small bowel wall, while they were not demonstrated in its outer parts. A difference in distribution of c-kit IR and CD34 IR cells in the small bowel wall was evident—c-kit IR cells did not exhibit simultaneous CD34 immunoreactivity. If the assumption of Huizinga and White [26] was applicable to the ICC development in humans, our findings would indicate that c-kit IR cells described in the small bowel wall in weeks 9–10 are in fact already differentiated, mature ICC. In other words, ICC precursors exhibit simultaneous c-kit and CD34 immunoreactivity, while mature ICC forms cease to exhibit CD34 and retain only c-kit IR properties [26]. On the other hand, according to the assumption by Horiguchi and Komuro [23], ICC precursors are c-kit negative and they could possibly appear as fibroblast-like cells. In that case, ICC precursors could be present in the small bowel wall in embryonal period of development, at 7–8 weeks, and the process of differentiation occurs at the beginning of foetal period when c-kit positive cells appear representing mature ICC. At the same time, the appearance of c-kit IR cells and longitudinal muscle layer is in accordance with the hypothesis by Klüppel et al. [19] who have claimed that ICC and smooth muscle arise from common precursor cells. Common precursors of the above cells are present in the outer parts of the bowel wall at the end of embryonic period of development, and later, at the beginning of fetal period of development a portion of these cells differentiates into smooth muscle cells of the longitudinal layer, while the rest differentiate into ICC. Certainly, the hypotheses stated above require further confirmation.

Conclusion

We may say that c-kit IR cells present in the small bowel wall at the beginning of fetal period of development, at 9–10 weeks, do not exhibit concurrent CD34 immunoreactivity.

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References

1. Komuro T. Structure and organization of interstitial cells of Cajal in the gastrointestinal tract. J Physiol 2006; 576:653–658.
2. Radenković G, Nikolić I, Todorović V. Interstitial cells of Cajal - pacemakers of the intestinal musculature. Facta Universitatis: Series Medicine and Biology 2005; 12:1–5.
3. Beckett EAH, Takeda Y, Yanase H, Sanders KM, Ward SM. Synaptic specializations exist between enteric motor nerves and interstitial cells of Cajal in the murine stomach. J Comp Neurol 2005; 493:193–206.
4. Thuneberg L, Peters S. Toward a concept of stretch-coupling in smooth muscle. I. Anatomy of intestinal segmentation and sleeve contraction. Anat Rec 2001; 262:110–124.
5. Thuneberg L. Interstitial cells of Cajal: intestinal pacemaker cells? Adv Anat Embryol Cell Biol 1982; 71:1–130.
6. Maeda H, Yamagata A, Nishikawa S. Requirement of c-kit for development of intestinal pacemaker system. Development 1992; 116:369–375.
7. Huizinga JD, Thuneberg L, Klüppel M, Malysz J, Mikkelsen HB, Bernstein A. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. Nature 1995; 373:347–349.
8. Ward SM. Interstitial cells of Cajal in enteric neurotransmission. Gut 2000; 47: 40–43.
9. Iino S, Horiguchi K. Interstitial cells of Cajal are involved in neurotransmission in the gastrointesinal tract. Acta Histochem Cytochem 2006; 39:145–153.
10. Thuneberg L, Peters S. Toward a concept of stretch-coupling in smooth muscle. I. Anatomy of intestinal segmentation and sleeve contraction. Anat Rec 2001; 262:110–124.
11. Maeda H, Yamagata A, Nishikawa S. Requirement of c-kit for development of intestinal pacemaker system. Development 1992; 116:369–375.
12. Radenkovic G, Savic V, Mitic D, Grahovac S, Bjelakovic M, Krstic M. Development of c-kit immunopositive interstitial cells of Cajal in the human stomach. J Cell Mol Med 2010a; 14:1125–1134.
13. Radenkovic G, Ilic I, Zivanovic D, Vlajkovic S, Petrovic V, Mitrovic O. C-kit-immunopositive interstitial cells of Cajal in human embryonal and fetal oesophagus. Cell Tissue Res 2010b; 340:427–436.
14. Radenkovic G. Two patterns of development of interstitial cells of Cajal in the human duodenum. J Cell Mol Med. 2012; 16:185-192.
15. Radenkovic G, Abramovic M. Differentiation of interstitial cells of Cajal in the human distal colon. Cells Tissues Organs 2012; 196:463–469.
16. Abramovic M, Radenkovic G, Velickov A. Appearance of interstitial cells of Cajal in the human midgut. Cell Tissue Res 2014; 356:9–14.
17. Burns AJ, LeDouarin NM. Enteric nervous system development: analysis of the selective developmental potentialities of vagal and sacral neural crest cells using quail-chick chimeras. Anat Rec 2001; 262:16–28.
18. Lecoin L, Gabella G, Le Douarin N. Origin of the c-kit-positive interstitial cells in the avian bowel. Development 1996; 122:725–733.
19. Klüppel M, Huizinga JD, Malysz J, Bernstein A. Developmental origin and Kit-dependent development of the interstitial cells of Cajal in the mammalian small intestine. Dev Dyn 1998; 211:60–71.
20. Lorincz A, Redelman D, Horvath VJ, Bardsley MR, Chen H, Ordög T. Progenitors of interstitial cells of Cajal in the postnatal murine stomach. Gastroenterology 2008; 134:1083–1093.
21. Pieri L, Vannucchi MG, Faussone-Pellegrini MS. Histochemical and ultrastructural characteristics of an interstitial cell type different from ICC and resident in the muscle coat of human gut. J Cell Mol Med 2008; 12:1944–1955.
22. Popescu LM, Faussone-Pellegrini MS. Telocytes - a case of serendipity: the winding way from interstitial cells of Cajal (ICC), via interstitial Cajal-like cells (ICLC) to telocytes. J Cell Mol Med 2010; 14:729–740.
23. Horiguchi K, Komuro T. Ultrastructural observations of fibroblastlike cells forming gap junctions in the W/W(nu) mouse small intestine. J Auton Nerv Syst 2000; 80:142–147.
24. Fu M, Tam PK, Sham MH, Lui VC. Embryonic development of the ganglion plexuses and the concentric layer structure of human gut: a topographical study. Anat Embryol 2004; 208:33–41.
25. Wallace AS, Burns AJ. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. Cell Tissue Res 2005; 319:367–382.
26. Huizinga JD, White EJ. Progenitor cells of interstitial cells of Cajal: on the road to tissue repair. Gastroenterology 2008; 134:1252–1254.

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PRES AS A COMPLICATION OF A MODERATE PREECLAMPSIA: CASE REPORT AND SHORT REVIEW OF LITERATURE

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Abstract. *We present a case of puerpera with two major complications of preeclampsia: PRES manifested by eclampsia and HELLP syndrome. PRES is neuroradiological entity characterized by hypertension, altered mental status, visual disturbances, headache and generalized seizures together with characteristic findings on cerebral magnetic resonance imaging scan. An important fact considering PRES (which also happened to our patient) is that it can be developed without significant rise in blood pressure, in a situation of severe endothelial injury with diminished cerebral autoregulatory capacity. Another consequence of vascular endotheliosis that developed in our patient was HELLP syndrome. Although the complications are severe, this state is usually completely reversible presuming that prompt diagnosis and adequate therapy were timely undertaken, as in the case we report here.*

Key words: *Eclampsia, posterior reversible encephalopathy syndrome, HELLP syndrome*

Introduction

Preeclampsia is a disorder that complicates 5–8% of all pregnancies [1–7]. Together with its most serious complications: pulmonary edema, acute renal failure, disseminated intravascular coagulation (DIC), syndrome of haemolysis, elevated liver enzymes and low platelets (HELLP), eclampsia, it is one of the leading causes of maternal and fetal morbidity and mortality (10–15% of all maternal deaths) [2, 7].

Genetic predisposition, immunologic intolerance between maternal and fetoplacental tissues and insufficient placentation lead to placental ischemia. This provokes abnormal nitric oxide and lipid metabolism, leukocyte and coagulation system activation, changes in various cytokines etc., resulting in generalized vasospasm and vascular endotheliosis [1, 2, 5, 6, 8–10]. Dominating symptom is hypertension, accompanied by significant proteinuria.

In this article we present a case of puerpera with two major complications of preeclampsia – posterior reversible encephalopathy syndrome (PRES), manifested by eclampsia, and HELLP syndrome.

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Case Report

A 23 years old primipara was admitted to our intensive care unit (ICU) 21 hours after spontaneous term delivery, when she gave birth to a live male child (3500 g / 53 cm, Apgar score 9) in her hometown hospital. First sixteen hours after delivery passed uneventfully; suddenly she lost conscience and suffered generalized tonic―clonic seizure. After a few minutes she regained consciousness, but remained disoriented. Blood pressure (BP) measured at that moment was 160/100 mm Hg. Ninety minutes later she suffered another seizure, after which she was transferred to our hospital.

On admission she was in the state of somnolence (during the transport she was given midasolam 45 mg), but oriented, able to adequately respond to our questions and obey commands. She claimed to be healthy till the day of the delivery, with no illness of any kind. She regularly visited her gynecologist during the pregnancy.

Physical examination on admission showed no pathological signs or symptoms, other than somnolence and hypertension – BP was 160/100 mm Hg, pulse rate 73 bpm. Venous blood samples were taken for hematological and biochemical analyses, and were repeated every day during her stay at our hospital.

On admission the results of the laboratory analyses out of the reference ranges were as follows: Hb 86 g/l, Ht 27%, Plt 70 × 10^9/l, indirect bilirubin 15.2 μmol/l, total proteins 49.5 g/l, albumins 22.3 g/l, ALT 74 U/l, AST 138 U/l, LDH 1339 U/l, serum Fe 83 μmol/l, CRP 46,9 mg/l.

24 hours later the results out of reference range were as follows: Hb 83 g/l, Ht 26.9%, Plt 103 x 10^9/l, total proteins 51 g/l, albumins 26 g/l, AST 71 U/l, LDH 716 U/l, serum Fe 21 μmol/l, haptoglobin 0.47 g/l; reticulocytes 10%, schistocytes on peripheral blood smear were found. The next day all results were in referent range, except Hb 75 g/l and Ht 24%.

Endocranial MSCT was done immediately after the admission: irregular, partly confluent hypodensity zones, in cortical and subcortical white matter that might correspond to PRES changes were detected in parieto-occipital regions bilaterally. Pons, cerebellum and mesencephalon showed no morphological changes. No presence of blood endocranially.

In order to get more subtle evaluation, magnetic resonance imaging (MRI) was done the next day (T1W sagittal, T2W/T2W FLAIR/ DW1 transversal and T2W coronal endocranial tomograms): zones with increased signal intensity, that correspond to PRES, were found in bilateral frontal, parietal and occipital regions in subcortical white matter (Figs. 1 and 2). There were no other pathological changes.

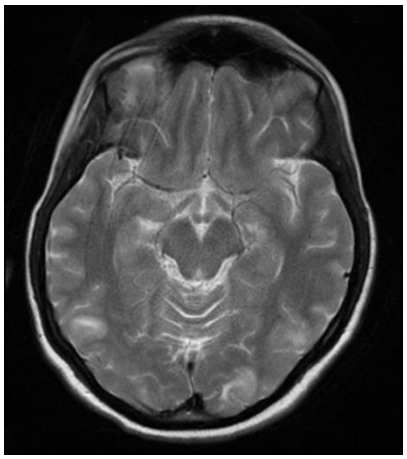

Fig. 1. Multiple cortico-subcortical areas of T2-weighted hyperintense signal involving the occipital lobes bilaterally in axial plane

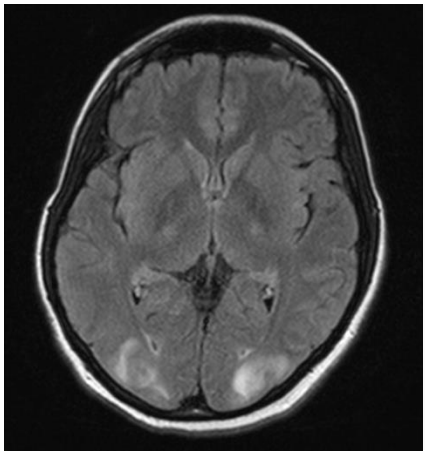

Fig. 2. Multiple cortico-subcortical areas of FLAIR-weighted hyperintense signal involving the occipital lobes bilaterally in axial plane

The patient was continuously monitored: BP, pulse rate, oxygen saturation, temperature, diuresis. All parameters were in normal range, except moderate to severe hypertension.

Antiedematous (sol. Manitholi 20% 125 ml/6h, dexamethasone 4 mg/8h), anticonvulsive (sol. $MgSO_4$ 1g/hour), antihypertensive, antibiotic, uterotonic and anticoagulant - low molecular weight heparin (LMWH) therapy was immediately started, as well as albumin, electrolyte and erythrocyte supplementation (according to laboratory results):

On the first day, there was one BP elevation to 170/110 mmHg, which was treated with urapidil 50 mg iv. The dose was sufficient to reduce BP to 130/80 mmHg, so we further proceeded with peroral therapy: Captopril 25 mg/12 hour which successfully kept BP between 120/80 and 140/90 mmHg.

During her stay at our Clinic, the patient had no eclamptic seizures, her laboratory results improved as well as her clinical state, so after five days she was transferred back to her hometown hospital.

Discussion

The presented case seems to be a good illustration of the complications that could be expected of the state we considered to be only the moderate form of preeclampsia. Preeclampsia can be manifested by wide range and intensity of symptoms – from severe hypertension and proteinuria, to mild or absent hypertension without proteinuria. According to recent data, 20% of women who developed eclampsia did not have any premonitory symptoms before the onset of convulsions; 16% of patients did not have diastolic blood pressure higher than 90 mmHg [11, 12].

Our patient (per anamnesis) did not have any obvious typical signs of preeclampsia till after the labour. Sixteen hours after the delivery her state deteriorated suddenly (with no prodromal signs) and she had two episodes of eclamptic seizures. Elevated blood pressure was registered for the first time. PRES was diagnosed on MRI scan.

PRES is a relatively new neuroradiological entity (first described by Hinchey at al. 1996.), characterized by clinical signs and symptoms of hypertension, altered mental status, visual disturbances, headache and generalized seizures together with characteristic findings on MRI scan [3, 13–20]. Besides eclampsia, PRES can occur in sepsis, after exposure to immunosuppressants, in autoimmune/renal/hypertensive diseases [13, 14, 21–24]. Underlying disorders in these conditions are immune system activation, inflammatory response, vascular instability and endothelial cell disfunction [13, 14, 17, 19, 22–24].

It is supposed that rapid rise in blood pressure, in a state of already compromised blood brain barrier (endothelial dysfunction) leads to breakthrough of cerebral blood flow autoregulation, forced dilatation of cerebral vessels, cerebral hyperperfusion and vasogenic cerebral edema [3, 14, 21, 23, 24]. Some findings,

though, suggest that neurological symptoms arise from "overautoregulation", vasospasm, hypoperfusion, that causes ischemia and, again, cerebral edema [14, 15, 21, 23–25]. Posterior brain is less innervated with sympathetic fibers that regulate cerebral blood flow, so parieto-occipital white matter is the region where the edema most frequently occurs, but changes can be also found in frontal lobes, basal ganglia, cerebellum, and brainstem [14, 17, 23]. T2 weighted MRI demonstrates regions of higher intensity, suggestive of vasogenic edema [13–17, 19–21,].

Very important fact considering PRES is that it can develop without a significant rise in BP [3, 14, 17, 22]. This is especially true in pregnancy, where the cerebral autoregulation curve is shifted to the lower range of BP [3, 14, 20, 26]. In cases of severe endothelial injury, as in preeclampsia, autoregulatory capability is completely diminished, so, as in our case, even a moderate elevation in BP (160/100 mmHg) can cause neurologic symptoms, culminating in eclamptic seizures. For that reason, in 2011, American College of Obstetricians and Gynecologists (ACOG) Committee [27] stated that: "Acute onset of severe systolic (more than 160 mmHg) or severe diastolic (110 mmHg) hypertension, or both, in pregnant women, persistent more than 15 minutes, is considered hypertensive emergency". The most important predictor of cerebral injury is systolic tension [3, 5, 25].

What is encouraging about PRES is that this state is usually completely reversible within 7 days, presuming that prompt control of seizures and BP and expeditious delivery (in cases of prepartal eclampsia) were undertaken [3, 13, 14, 18, 21, 25]. Our therapy regimen included antiedematous therapy (sol. Manithol 20% 125 ml/6h, Dexamethasone 4 mg/8h) and anticonvulsive therapy (Magnesium sulfate infusion 1 g/h). Magnesium is considered the drug of choice for seizure prophylaxis and control in eclapmsia [1–3, 7, 17, 23, 26–28]. Because of toxic effects of hypermagnesemia (cardiac arrhythmia and respiratory depression), magnesemia, diuresis and deep tendon reflexes should be closely monitored.

Antihypertensive therapy in pregnancy and lactation is highly limited, because of drug effects on the fetus as well as on maternal circulation. Sudden reduction of BP can compromise fetal-placental circulation, so, even in cases of emergency, only 10% BP reduction is recommended during first hour and another 15% gradually over next 2–3h [29–33]. The first choice drug is intravenous labetolol, followed by hydralazine, nicardipine (in cases of cerebral vasospasm), sodium nitroprusside (only in most refractory cases), nitroglycerin (especially in pulmonary edema) and nifedipine per os [2, 4, 6, 7, 18, 26, 28–37]. Urapidil is allowed in pregnancy as well and it seemed to be a good choice in case of our patient, when raised intracranial pressure was presumed and cerebral vasodilatation was to be avoided [35, 36]. The urapidil dose of 50 mg was effective enough and allowed us to switch to peroral therapy with captopril 25 mg/12h. ACE inhibitors are contraindicated in pregnancy, but enalapril and captopril are allowed during lactation [5, 6, 10, 32, 34–36]. Considering the fact that renin–angiotensin–aldosteron system is also affected in preeclampsia, we chose captopril and had a satisfactory effect.

The same patient suffered from another consequence of damaged vascular endothelium: syndrome of hemolysis (LDH level over 600 IU/l), elevated liver enzymes (AST and ALT levels higher than 70 IU/l) and low platelet count (below 150×10^9 /l) – HELLP syndrome [2, 7, 38, 39]. HELLP is a complication in 10–15% cases of eclampsia and up to 20% of cases of early onset antepartum eclampsia [12, 40, 41]. It represents a serious complication of pregnancy that might cause maternal acute renal failure, peripartal hemorrhage, and fetal intrauterine growth restriction (IUGR), fetal thrombocytopenia, hemorrhage and death. HELLP demands prompt fetal delivery and removal of the placenta (as placenta is the primary cause of toxemia). Our patient had all diagnostic parameters of HELLP on admission. Hemolysis was proven by elevated LDH levels. Low haptoglobin level is even better marker of hemolysis; in our case the level was near lower limit. It would have been interesting to know her previous results, but unfortunately they were not available. Symptoms of the disease resolved rather quickly. We presume that it was (besides the fact that pregnancy was terminated and placenta, the main cause of the disease, removed) partly because of prompt treatment and may be because it was the case of late onset preeclampsia, which has a more favorable clinical course compared to the early onset preeclampsia.

Conclusion

We emphasized that it is important to keep in mind that eclampsia can develop even with moderate elevation of BP. If PRES is not adequately diagnosed and treated, ischemic cerebral injury and irreversible neurological damage could develop, as well as impaired cognitive function later in life. With prompt seizure and BP control, the process is completely reversible.

References

1. Sibai BM. Diagnosis and management of gestational hypertension and preeclampsia. Obstet Gynecol 2003; 102: 181–192.
2. Turner JA. Diagnosis and management of preeclampsia: an update. Int J Womens Health 2010; 2:327–337.
3. Wagner SJ, Acquah LA, Lindell PE, et al. Posterior reversibile encephalopathy syndrome and eclampsia: pressing the case for more aggressive blood pressure control. Mayo Clin Proc 2011; 86:851–856.
4. Winer N, Tsasaris U. État des connaissances: prise en charge thérapeutique de la prééclampsie. J Gynecol Obstet Biol Reprod (Paris) 2008; 37:5–15. (French)
5. Magee LA, Helleva M, Moutquin JM, von Dadelszen P. Diagnosis, evaluation and management of the hypertensive disorders of pregnancy. J Obstet Gynaecol Can 2008; 30:S1–48.

6. Chandiramani M, Waugh JS, Shennan AH. Management of hypertension and pre-eclampsia in pregnancy. Trends Urol Gynaecol Sex Health 2007a; 12:23–28.
7. Brodie H, Malinow M. Anesthetic management of preeclampsia/eclampsia. Int J Obstet Anesth 1999; 8:110–124.
8. Borzychowski AM, Sargent IL, Redman CW. Inflammation and pre-eclampsia. Semin Fetal Neonatal Med 2006; 11:309–316.
9. Staff AC, Dechend R, Pijnenborg R. Learning from the placenta: acute atherosis and vascular remodeling in preeclampsia-novel aspects for atherosclerosis and future cardiovascular health. Hypertension 2010; 56:1026–1034.
10. Visintin C, Mogglestone MA, Almerie MQ, Nherera LM, James D, Walkinshaw S. Management of hypertensive disorders during pregnancy: summary of NICE guidance. BMJ 2010; 341:c2207. http://www.bmj.com/content/341/bmj.c2207
11. Staykov D, Schwab S. Posterior reversible encephalopathy syndrome. J Intensive Care Med 2012; 27:11–24.
12. Sibai BM. Diagnosis, preventions and management of eclampsia. Obstet Gynecol 2005; 105:402–410.
13. Mishra SK, Bhat R, Sudeep K, Nagappa M, Swain A, Badhe A. PRES (posterior reversible encephalopathy syndrome) and eclampsia: Review. The Internet J Anesth 2009; 22(1). https://ispub.com/IJA/22/1/13690
14. Cipolla MJ. Cerebrovascular function in pregnancy and eclampsia. Hypertension 2007; 50:14–24.
15. Nakagawa K, Sorond FA, Ropper AH. Ultra-early magnetic resonance imaging findings of eclampsia. Arch Neurol 2008; 65:974–976.
16. Holdcroft A, Oatridge A, Fusi L, Hajnal JV, Saeed N, Bydder GM. Magnetic resonance imaging in preeclampsia and eclampsia complicated by visual disturbance and other neurological abnormalities. Int J Obstet Anesth 2002; 11:255–259.
17. Nuwer JM, Eshaghian S. Late postpartum eclampsia with posterior reversibile encephalopathy syndrome. Hospital Physician 2007; pp 45–49. http://www.turner-white.com/memberfile.php?PubCode=hp_jun07_late.pdf
18. Powell ES, Goldman MJ. Posterior reversibile encephalopathy syndrome (PRES) in a thirty-six-week gestation eclamptic. J Emerg Med 2007; 33:377–379.
19. Bartynsky WS. Posterior reversibile encephalopathy syndrome, part 1: fundamental imaging and clinical features. AJNR Am J Neuroradiol 2008; 29:1036–1042.
20. Prout RE, Tuckey JP, Giffen NJ. Reversible posterior leuceoencephalopathy syndrome in peripartum patient. Int J Obstet Anesth 2007; 16:74–76.
21. Fugate JE, Claassen DO, Cloft HJ, Kallmes DF, Kozak OS, Rabinstein AA. Posterior reversible encephalopathy syndrome: associated clinical and radiologic findings. Mayo Clin Proc 2010; 85:427–432.
22. Bartinsky WS, Bordman JF, Ziegel RR, Shadduck RK, Lister J. Posterior reversibile encephalopathy syndrome in infection, sepsis and schock. AJNR Am J Neuroradiol 2006; 27:2179–2190.
23. Bartynsky WS. Posterior reversible encephalopathy syndrome, part 2: controversies surrounding pathophysiology of vasogenic edema. AJNR Am J Neuroradiol 2008; 29:1043–1049.
24. Brubaker LM, Smith JK, Lee YZ, Lin W, Castillo M. Hemodynamic and permeability changes in posterior reversible encephalopathy syndrome measured by dynamic susceptibility perfusion-weighted MR imaging. AJNR Am J Neuroradiol 2005; 26:825–830.
25. Onrubia X, Lluch-Oltra A, Armero R, Higueras R, Sifre C, Barbera M. Posterior reversible encephalopathy syndrome after a cesarean delivery. Anesth Analg 2007; 104:746–747.
26. Vaughan CJ, Delanty N. Hypertensives emergencies. Lancet 2000; 356:411–417.
27. American College of Obstetricians and Gynecologosts Committee opinion no. 514: emergent therapy for acute-onset, severe hypertension with preeclampsia or eclampsia. Obstet Gynecol 2011; 118:1465–1468.
28. Haas AR, Marik PE. Current diagnosis and management of hypertensive emergency. Semin Dial 2006; 19:502–512.
29. Feldstein C. Management of hypertensive crysis. Am J Ther 2007; 14:135–139.
30. Marik PE, Varon J. Hypertensive cryses: challenges and management. Chest 2007; 131:1949–1962.
31. Hardy YM, Jenkins AT. Hypertensive crysis: urgencies and emergencies. US Pharmacist 2011; 36(3). http://www.uspharmacist.com/content/d/feature/i/1444/c/27112/
32. Chandiramani M, Shennan J. Waugh JJS. Modern management of postpartum hypertension. Trends Urol Gynaecol Sex Health 2007b; 12:37–42.
33. Cline DM, Amin A. Drug treatment for hypertensive emergencies. New concepts and emerging technologies for emergency physicians. EMCREG-Int 2008; 1:1–11. http://www.emcreg.org/pdf/monographs/2008/Cline2007.pdf
34. Rakusan K. Drugs in pregnancy: implications for a cardiologist. Exp Clin Cardiol 2010; 15:e100–103.
35. Podymow T, August P. Update of the use of antihypertensive drugs in pregnancy. Hypertension 2008; 51:960–969.
36. Habek D, Moslavac S, Čerkez-Habek J. Lječenje hipertenzivne bolesti u trudnoći. Med Jad 2011; 41:23–35. (Croatian)
37. RCOG Guideline No 10(A). The management of severe preeclampsia and eclampsia. 2010; pp 1–11. http://isshp.org/wp-content/uploads/2014/05/rcog.pdf
38. Haram K, Svendsen E, Abildgaard U. The HELLP syndrome: clinical issues and management. A review. BMC Pregnancy & Childbirth 2009; 9(8). http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2654858/
39. Leeman L, Fontaine P. Hypertensive disorders in pregnancy. Am Fam Physician 2008; 78:93–100.
40. Mammaro A, Carrara S, Cavaliere A, et al. Hypertensive disordes of pregnancy. J Prenat Med 2009; 3:1–5.
41. Mattar F, Sibai BM. Eclampsia. VIII. Risk factors for maternal morbidity. Am J Obstet Gynecol 2000; 182:307–312.

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TETRALOGY OF FALLOT: REPORT OF TWO CASES IN OUTPATIENT PRACTICE

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Abstract. *Tetralogy of Fallot or Tetras Fallot is a congenital heart disease with cyanosis, which involves four associated anomalies of the heart. As physicians in primary health care of children, we treat and monitor the growth and development of children at risk with special attention. Among them are also the children with tetralogy of Fallot. The aim of this report was to present and compare postnatal status of two children with tetralogy of Fallot. We presented the girl and a boy, approximately the same age, operated at the same surgical clinic. Both patients were without hereditary load. The girl had a milder clinical course without complications. The boy had associated anomalies, cerebrovascular insult, and a reoperation. After complete surgical correction of congenital heart disease, both children are progressing and developing within the normal range for age. ECG of both children showed the signs of right bundle branch block. Our mission is to continue to closely monitor these children and react in case of complications or worsening of recent findings.*

Key words: *Tetralogy of Fallot, postnatal course, pediatric patient, outpatient care*

Introduction

Tetralogy of Fallot (TOF) or Tetras Fallot is cyanogen, congenital heart disease with the right-to-left shunt. TOF includes combination of four associated heart anomalies: right ventricular outflow tract obstruction (stenosis of pulmonary trunk), ventricular septal defect (VSD), right ventricular hypertrophy and "overriding" aorta [1–7].

Embryologically, these anomalies occur as a result of disorders in morphogenesis of the right ventricle infundibulum [1]. In this congenital heart disease, infundibular septum is moved anterosuperior [1, 8], resulting in a large defect of the membranous part of the septum, immediately below the aortic valve, and movement of the aortic confluence to the right, and consequently, narrowing of the pulmonary tree. Aorta is dilated [4, 9, 10] and communicates with both ventricles ("overriding" aorta). Aortic arch was oriented to the right in 15–25% of patients [2, 4, 7, 11], without hemodynamic significance [2].

Ventricular septal development is a complex process that includes different septal structures of various origins and different positions. Ventricular septum is formed from the 5^{th} to the end of the 7^{th} week of embryonic development, by merging muscular septum with ventricular outflow tract and aortopulmonary septum [12].

The incidence of TOF is 0.4/1000 of live births [1, 13]. Compared to all congenital heart diseases, presented by various authors, the incidence of TOF ranged from 3–5% [3, 4, 8, 14], to 7–10% [1, 2, 9]. TOF representation in boys and girls was almost in the same range [3, 16], with a slightly higher percentage among boys (1.56:1) [15].

Etiology of TOF is multifactorial, and it is considered that the genetic factors, in combination with environmental conditions are responsible for developing this heart anomaly [2, 17, 18]. Recent studies have indicated that the TOF is often associated with deletion on chromosome 22—22q11 deletion, as in DiGeorge [2, 3, 7, 19] and Shprintzen (velo-cardial-facial) syndromes [3, 7, 19]. It was also indicated that there is a relationship between the congenital heart disease and the level of the vascular endothelial growth factor, considering that its concentration was elevated in children with cyanotic heart disease [20].

Diagnosis is based on anamnestic data, and clinical examination (central type of cyanosis, heart murmurs, fatigue, dyspnea and occurrence of paroxysmal cyanotic crises, development of clubbing fingers, slow growth, chest deformity), chest rentgenography, electrocardiogram (ECG), echocardiography, laboratory analysis, cardiac catheterization (in candidates for surgery), computer tomography of the heart, aortography and coronary arteriography [1–4, 7], and recently, magnetic resonance imaging [4].

Anomalies associated with TOF have been described and these include the ASD [1, 2, 7, 10, 11, 21], or multiple VSD [7], or congenital absence of pulmonary valve [7, 16, 21]. In the older literature [1, 10], TOF associated with ASD has been labeled as Pentalogy of Fallot.

The aim of this study was to present subjective and objective status of two children with TOF operated at the same surgical clinic and to compare their preoperative and

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postoperative course with the cases of (inter)national practice.

Material and Methods

Presented data were based on anamnesis, physical examination, ultrasound findings, data from medical records and discharge papers of the two children of male and female gender and similar age.

Ethical code has been respected; the author obtained the written consent from the parents (mothers) of both children for scientific presentation of children's medical records.

Case Reports

Case 1 (8.5 years old girl). It is the second child from the (second) normal pregnancy. Both parents and older sister are in good health, and without congenital heart disease. The child was born at term in a natural way. At birth she was blue, started crying later, body weight (BW) was 3800 g and the Apgar score 9. TOF was diagnosed at birth.

In the first year of life, the girl did not develop properly. Mild cyanosis was noticed in the third month of life, auscultation revealed systolic murmur intensity IV/6. The mother noticed that the girl was tired while feeding. Laboratory findings of erythrocytes, hematocrit and hemoglobin were within the normal range. The girl had upper respiratory infections several times and one hospitalization due to lower respiratory infection. The girl was not indicated for cardiac medicamentous therapy until the term for surgery.

Complete surgical reparation was performed at the age of one year. Diagnostic methods — echocardiography and heart catheterization, performed preoperatively, indicated the following abnormalities: non-restrictive perimembranous defect was positioned on the interventricular septum, over which the overriding aorta was about 50%, the right ventricle was hypertrophic, and right ventricular outflow tract was narrowed in the infundibular level with thick muscular wall. Aortic arch was described as "left", without anomaly.

A large perimembranous VSD was determined intraoperatively; it was 12–14 mm in diameter, with overriding aorta over 50%, fibro-muscular obstruction of the infundibulum, and severe hypertrophy of the right ventricle.

The postoperative period was normal. The girl progressed well in weight and height; echocardiography showed normal findings, regurgitation was registered at the root of the pulmonary artery, but it was hemodynamically insignificant. Auscultation sounds were clear, with normal intensity, without murmurs, and the ECG findings pointed to a right bundle branch block with sinus rhythm.

The author of this work regularly monitored the girl since 2011. During this time the girl had six upper respiratory infections, inflammation of the middle ear three times, and flu (influenza) but without complications. During follow-up by the author, she did not have inflammation of lower respiratory tract; according to data from medical record, one year after surgery, she had bronchitis, which was complicated with pneumonia. Systematic examination for school enrollment indicated that her body weight corresponded to the average values for age, while her body height was below average, but still within normal values. Blood elements were within the normal range, as well as blood pressure.

Her recent (July 2014) ECG is presented here (Fig. 1).

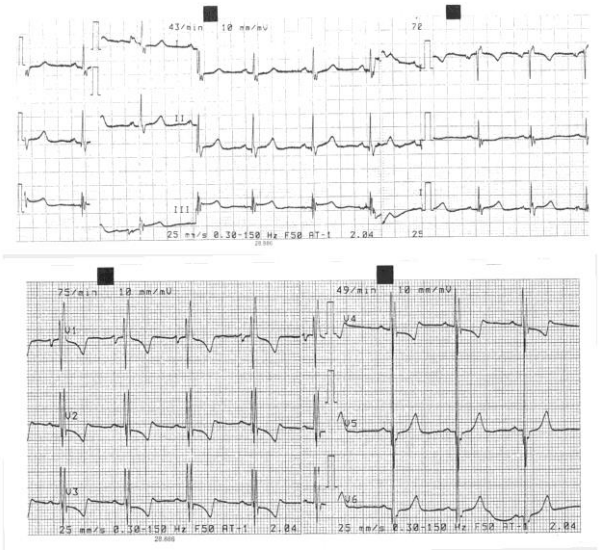

Fig. 1. Sections of ECG of the girl. The girl's ECG: normal axis; signs of the incomplete right bundle branch block in leads V_1–V_4 (presence of 2 R waves - RSR^1; QRS 0.08 s); negative T waves in leads V_1–V_4.

Case 2 (8 years old boy). He is the firstborn, bigger child (in body weight) from a twin pregnancy. Birth was premature — in the 36th week, however the Apgar score was 9. Diagnosis — Pentalogy of Fallot associated with bilateral inguinal hernia was determined at birth.

In the first month of life there were mild cyanosis, signs of anemia and systolic-diastolic murmur intensity IV/6. Therapy included iron containing medication. One month later, blood elements were within the normal range, but peripheral cyanosis and the same heart murmur were still present. In the third month, during the regular check-up, the mother stated that the child had severe cyanosis after defecation and during intensive crying. In the fourth month of life the crisis of cyanosis and vomiting occurred, and that was the reason why the child was admitted to hospital; the drug Propranolol has been introduced and the child was prepared for surgery. Preoperative examination confirmed larger right side of the heart than the left, large perimembranous outlet VSD and overriding aorta with left-oriented arch, infundibular and valvular stenosis of the pulmonary artery, and large ASD secundum.

During the cardiac catheterization the boy fell into hypotension, hypoxemia and consequently developed

cerebrovascular insult (CVI). Several days later, a neurological deficit has developed, presented with paresis of the left half of the body; which was partially withdrawn for two weeks. In the process of another preparation for surgery, the drug Propranolol was discontinued. However, two days later, at first mild, and then severe crises of cyanosis and loss of consciousness appeared. Propranolol was reintroduced, while the Medical Consilium decided to immediately perform a complete surgical correction.

Intraoperative diagnosis determined a large infracaval ASD (missed the entire bottom edge of the interatrial septum), a large perimembranous VSD with about 12 mm in diameter, overriding aorta (> 60%), while the infundibulum of the right ventricle was very narrow, almost like atresia, however, the process of fibrosis was not being expressed.

The postoperative course was normal until the 6thday, when the crises of cyanosis occurred again, accompanied by extreme hypertension (more than 200 mm Hg). Echocardiography pointed to residual VSD and significant tricuspid insufficiency. It was followed by reintervention and after that, the boy's condition stabilized.

In the further course, the boy was followed by a cardiologist and a neurologist. Auscultation revealed systolic murmur intensity III/6 along the left edge of the sternum. Neurological examination registered the phenomenon of "sunset", the occasional eye deviation to the left, hypotonia of the axis of the body and upper extremities and that boy could not to sit spontaneously and his reflexes were enhanced. Over time, the boy progressed in body weight and height and gradually recovered neurologically.

The boy had the first infection of the airways (pneumonia) at the age of eight months. By the age of 8 years he had bronchitis nine times and pneumonia three times.

Inguinal hernias, diagnosed at birth, were operated at the age of 2.5 years.

At the systematic examinations, starting from the third month of life until the age of seven, the boy was shorter in height, with lower body weight compared to his twin brother. There was considerable discrepancy in the growth and development from the age of three months to one year, but even after that period, the boy was in a certain disadvantage compared to his twin brother, who had a lower birth weight.

The author of this study followed and treated the boy since 2009. During this time the boy had six lower respiratory tract infections, without pneumonia. The systematic examination upon school enrollment showed that the boy's weight and height corresponded to the average values for age, auscultation of the heart indicated a systolic murmur intensity IV/6, blood elements were in the normal range, as well as the blood pressure.

His recent (September 2014) ECG is presented here (Fig. 2).

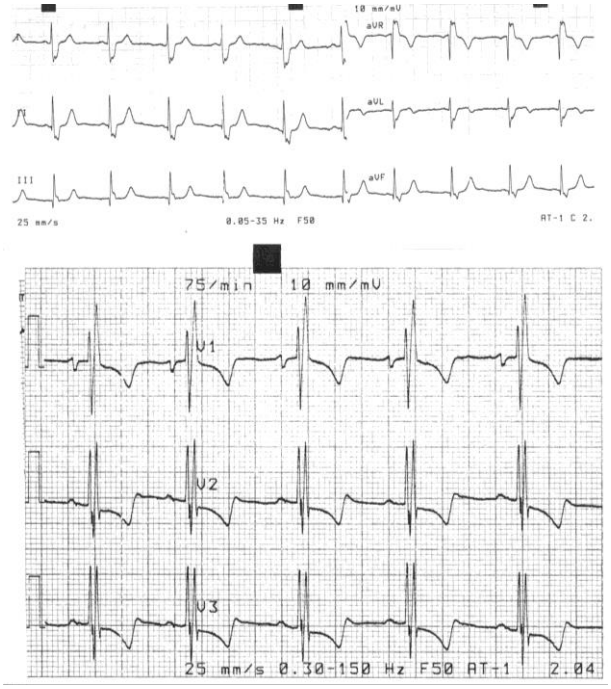

Fig. 2. Sections of EGG of the boy. The boy's ECG: normal axis, signs of the complete right bundle branch block in leads V_1–V_3 (presence of 2 R waves - RSR^1; QRS 0.132 s); there are no other rhythm disorders.

Discussion

We presented two children of similar age with TOF — one girl (from the second pregnancy) and one boy (from twin pregnancy). Both children were without hereditary load. However, the boy, according to the literature, had the risk of "twin process" [19]. The literature describes cases of twin pregnancies in which only one of the twins had a TOF [2, 10, 14, 19]. In addition to possible genetic causes, this is also explained by the previously mentioned "twin process", where blood flow is greater in one twin, while the other has less blood flow, which can cause the occurrence of congenital heart defects. Such a twin is smaller [5, 19], with lower body weight (BW) and body height, which was not the case with our little boy, who was the heavier twin at birth.

Our boy also had associated anomalies —ASD and bilateral inguinal hernia. According to the literature, TOF associated with ASD was found in 2% of those patients. Only in the older literature [1, 10], TOF associated with ASD has been labeled as Pentalogy of Fallot.

In these patients, symptoms and treatments are the same as those with TOF, but surgical correction and postoperative course are more complex [2], which was also the case with our patient. Inguinal hernia, as an extracardiac anomaly, associated with TOF is also described in the literature [5, 14].

In the first three months of life gradual appearance of cyanosis in both children was observed, which was objectively confirmed in other children as well [2, 7, 8].

Also, the crises of cyanosis, which typically begin to appear a few months after the birth during the child's agitation (crying) and decreased hydration [2], were observed in our boy in the fourth month of life. Temporary introduction of Propranolol in the treatment of cyanotic crises, according to the literature, is justified [2, 3, 7].

For the girl who had no associated anomalies and in whom the symptoms were mild, surgical correction of TOF was planned and performed at the age of one year. With the boy, surgical correction was performed at the age of 6 months, because the attacks of cyanotic crises became stronger. Age at which both corrections were done and the fact that complete surgical corrections of heart defects were performed, without prior palliative interventions, is in line with the attitude and experience of authors of recent (inter) national literature [2–4, 8, 11, 13, 22, 23]. In preparation for surgery, the boy had CVI, which briefly delayed the operation. In the available literature, there is evidence that 1.5% of patients had CVI before surgery TOF [15].

The postoperative course for the girl was without complications, while the boy had a complicated postoperative course because of reoperation and the previous stroke. After surgery, both children progressed well, to some degree compensating the backlog of BW and height and continue to have development within the normal range for their age. Such favorable postoperative course and favorable prognosis for life are indicated by many authors [2, 11, 13, 16, 24, 26].

The right bundle branch block, presented in ECG, is a common finding in patients after TOF surgery [11]. According to available data, incomplete right bundle branch block after surgery was present in about 20.2% of patients [25], while complete right bundle branch block, according to various authors was present in 65% [25] or 88% [15], or even 94% [26].

Conclusion

We presented the postnatal course of TOF in 8-years-old girl and boy. After a complete surgical correction, both children developed within the normal range for age.

Our mission in outpatient practice is to continue to closely monitor these children, to control regularly their health status, ECG and blood pressure and react promptly in case of complications or worsening of recent findings.

References

1. Josipović Z. Urođene mane srca sa desno-levim šantom. In: Stojmirović E, Popović-Rolović M, Nedeljković V (eds) Pedijatrija. Savremena administracija: Beograd, 1993; pp 570–574. (Serbian)
2. Baillard F, Anderson RH. Tetralogy of Fallot. Orphanet J Rare Dis 2009; 4:2. http://www.ojrd.com/content/4/1/2
3. Apitz C, Webb GD, Redington AN. Tetralogy of Fallot. Lancet 2009; 374:1462–1471.
4. Fox D, Devendra GP, Hart SA, Krasuski RA. When "blue babies" grow up: what you need to know about tetralogy of Fallot. Cleve Clin J Med 2010; 77:821–828.
5. Boon AR, Farmer MB, Roberts DF. A family study of Fallot's tetralogy. J Med Genet 1972; 9:179–192.
6. Guyton AC. Medicinska fiziologija. Medicinska knjiga: Beograd-Zagreb, 1988. (Serbian)
7. Berstein R. Tetralogy of Fallot. In: Kleigman RM, Stanton BF, St Geme III JW, Schor NF, Behrman RE (eds) Nelson textbook of pediatrics. Saunders: Philadelphia, 2011; pp 1573–1578.
8. Poon LCY, Huggon IC, Zidere V, Allan LD. Tetralogy of Fallot in the fetus in the current era. Ultrasound Obstet Gynecol 2007; 29:625–627.
9. Tan JL, Davlouros PA, McCarthy KP, Gatzoulis MA, Ho SY. Intrinsic hystological abnormalities of aortic root and ascending aorta in tetralogy of Fallot: evidence of causative mechanism for aortic dilatation and aortopathy. Circulation 2005; 112:961–968.
10. Lillehei CW, Cohen M, Warden HE, et al. Direct vision intracardiac surgical correction of the tetralogy of Fallot, pentalogy of Fallot, and pulmonary atresia defect; reports of first ten cases. Ann Surg 1955; 142:418–442.
11. Bacha EA, Scheule AM, Zurakowski D, et al. Long-term results after early primary repair of tetralogy of Fallot. J Thorac Cardiovasc Surg 2001; 122:154–161.
12. Abdulla R, Blew A, Holterman MJ. Cardiovascular embryology. Ped Cardiol 2004; 25:191–200.
13. Bertranou EG, Blackstone EH, Hazelrig JB, Turner ME, Kirklin JW. Life expectancy without surgery in tetralogy of Fallot. Am J Cardiol 1978; 42:458–466.
14. Mitchell SC, Korones SB, Berendes HW. Congenital heart disease in 56.109 births: incidence and natural history. Circulation 1971; 53:323–332.
15. Garson A Jr, Nihill MR, McNamara DG, Cooley DA. Status of the adult and adolescent after repair of tetralogy of Fallot. Circulation 1979; 59:1232–1240.
16. Lakier JB, Stanger P, Heymann MA, Hoffman JIE, Rudolph AM. Tetralogy of Fallot with absent pulmonary valve: natural history and hemodinamic considerations. Circulation 1974; 50:167–175.
17. Nora JJ. Multifactorial inheritance hypothesis for the etiology of congenital heart diseases: the genetic-enviromental interaction. Circulation 1968; 38:604–617.
18. Kostić SM. Urođene srčane mane. In: Stojmirović E, Popović-Rolović M, Nedeljković V (eds) Pedijatrija. Savremena administracija: Beograd, 1993; pp 557–558. (Serbian)
19. Goodship J, Cross J, Scambler P, Burn J. Monozygotic twins with chromosome 22q11 deletion and discordant phenotype. J Med Genet 1995; 32:746–748.
20. Bahtiyar MO, Dulay AT, Weeks BP, Friedman AH, Copel JA. Prevalence of congenital heart defects in monochorionic/diamniotic twin gestations. J Ultrasound Med 2007; 26:1491–1498.
21. Chrysostomou C, Tsifansky M, Morell VO. Tetralogy of Fallot with absent pulmonary valve. In: Munoz R, Morell V, Cruz E, Vetterly C (eds) Critical care of children with heart disease: basic medical and surgical concepts. Springer-Verlag: London, 2010; pp 207–211.
22. Sullivan ID, Presbitero P, Gooch VM, Aruta E, Deanfield JE. Is ventricular arrhythmia in repair tetralogy of Fallot an effect of operation or a consequence of the course of the disease? Br Heart J 1987; 58:40–44.
23. Pigula FA, Khalil PN, Mayer JE, del Nido PJ, Jonas RA. Repair of tetralogy of Fallot in neonates and young infants. Circulation 1999; 100:II-157-II-161.
24. Harrison DA, Harris L, Siu SC, et al. Sustained ventricular tachycardia in adult patients late after repair of tetralogy of Fallot. J Am Coll Cardiol 1997; 30:1368–1373.
25. Quattlebaum TG, Varghese J, Neill CA, Donahoo JS. Sudden death among postoperative patients with tetralogy of Fallot: a follow-up study of 243 patients for an average of twelve years. Circulation 1976; 54:289–293.
26. Gillette PC, Yeoman MA, Mullins CE, McNamara DG. Sudden death after repair of tetralogy of Fallot. Electrocardiographic and electrophysiologic abnormalities. Circulation 1977; 56:566–571.

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PSEUDOPARASITIC ACUTE APPENDICITIS. REPORT OF A RARE CASE

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Abstract. *The etiology of acute appendicitis is multifactorial with fecal stasis and fecaliths being the most common causes. The etiopathogenetic role of parasitic infection in acute appendicitis is still debatable. Appendiceal parasite and/or its ova may produce intraluminal obstruction resulting in acute appendicitis or lead to a secondary inflammation. Nevertheless, luminal obstruction of the appendix may be caused by numerous materials of different origin many of which may resemble parasitic infestation on pathology analysis thus qualifying as pseudoparasitic inflammation. Therefore, pathological examination of removed appendices must be careful and thorough to confirm real parasitic acute appendicitis or recognize pseudoparasitic inflammation and, if necessary, supplemented with stool examination for parasitic infection.*

Key words: *Acute appendicitis, intestinal parasites*

Introduction

Acute appendicitis (AA) is the most frequent urgent abdominal surgical condition [1]. The etiology of appendiceal obstruction and consequent AA is multifactorial with fecal stasis and fecaliths being the most common causes [2]. This may explain higher incidence of AA in industrialized communities where the low-fiber diet is predominantly consumed [3]. Although the etiopathogenetic role of parasitic infection in AA has been discussed for more than 100 years [4], there is still not enough evidence regarding the relationship between these two entities.

Since the luminal obstruction of the appendix may be caused by numerous materials of different origin many of which may present as (pseudo)parasitic infestation on pathology analysis, the aim of this paper is to present a patient with such condition.

Case Report

A 46-year old man was admitted to our department for severe pain in lower right abdominal quadrant accompanied with fever (38.7°C) and nausea. The symptoms started the previous evening when he experienced sudden nausea and vomited twice without relief. This was followed by mild pain in the epigastrium, which after few hours, migrated to lower right abdomen, was continuous and characterized by slow but progressive increase of severity. On physical examination, rebound tenderness in right iliac region was found, laboratory investigation revealed leukocytosis (16×10^9/L) and elevated serum C-reactive protein level (110mg/L) and there was fever with 1.1°C variation between axillary and rectal body temperature (37.6°C and 38.7°C respectively).

The diagnosis of AA was made and the patient underwent surgery. Intraoperatively, phlegmonous appendicitis and purulent periappendiceal inflammation was found.

Macroscopic diagnosis was confirmed on pathology report which revealed the appendix luminal obstruction with parasite-like or pseudoparasitic structures (Figs. 1 and 2).

Postoperative course was uneventful. Peristalsis followed by normal stool occurred on the 4th postoperative day. After discharge from hospital, (triple) fecal examination for parasitic infection was negative.

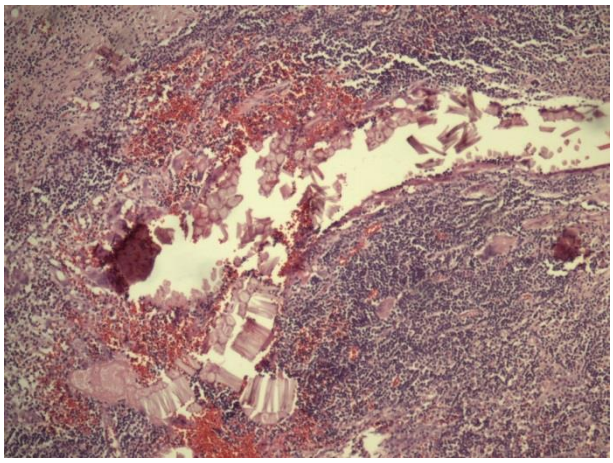

Fig. 1. Pseudoparasitic (parasite-like) structures in appendiceal lumen (HE staining × 200).

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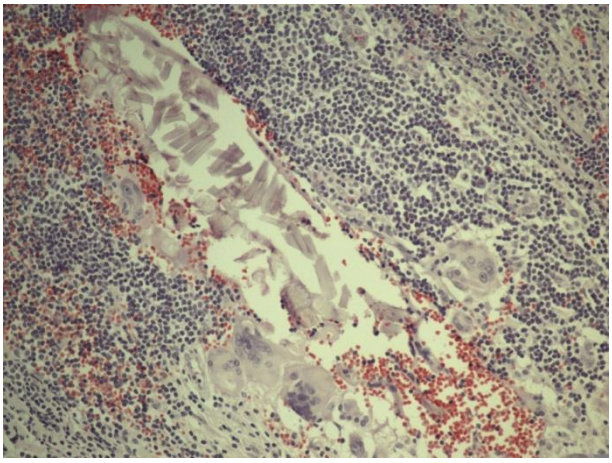

Fig. 2. Acute appendicitis with giant cells in close relation (surrounding) to pseudoparasitic structures (HE staining × 200).

Discussion

It is considered that the lifetime risk of AA is approximately 6-7% with a peak incidence in the second and third life decades [1, 2, 5]. Appendiceal lumen obstruction which precedes acute inflammation is most often caused by fecal stasis and fecaliths. Other causes include lymphoid hyperplasia, vegetable matter, fruit seeds, barium radiographic contrast, intestinal parasites and tumors [2]. Intraluminal pressure increase due to obstruction, leads to vascular congestion, mucosal ischemia and ulcerations. Mucosal barrier is compromised and appendiceal wall is invaded by intraluminal bacteria, which is furthermore fueled by intraluminal bacteria overgrowth due to stasis.

Parasitic infestation is a very rare cause and has a debatable role in the pathogenesis of AA [6]. Increased incidence of intestinal parasitic infection in tropical countries is not associated with more common appediceal inflammation [7]. Appendiceal parasite and/or its ova may produce intraluminal obstruction resulting in AA or lead to a secondary inflammation. Nevertheless, very often the symptoms of appendiceal parasitic infestation may mimic those of AA without really causing it [7]. That is why the majority of parasitic infestations of appendix is not associated with an acute inflammation and is considered to be a component of false AA [4]. The parasite and/or its ova in the appendix may cause recurring appendiceal colic and abdominal discomfort due do luminal obstruction and wall distension without eliciting an acute inflammation which may result in multiple visits to hospital and eventually mislead to the diagnosis of AA and surgery [8]. Also, many of numerous causes of appendiceal intraluminal obstruction may resemble parasitic infestation on microscopic examination thus falsely presenting parasites as a cause of AA, like in the presented case, and qualifying as pseudoparasitic inflammation.

Conclusion

An appendiceal colic caused by parasitic infestation most often cannot be differentiated from lower right abdominal pain typical for AA and often leads to surgery. Nevertheless, the majority of patients with appendiceal parasites and such symptoms do not experience an acute inflammation of the appendix. Pathological examination of removed appendices must be careful and thorough to confirm real parasitic AA or recognize pseudoparasitic inflammation and, if necessary, supplemented with stool examination for parasitic infection.

References

1. Humes DJ, Simpson J. Acute appendicitis. Br Med J 2006; 333:530–534.
2. Flasar MH, Goldberg E. Acute abdominal pain. Med Clin North Am 2006; 90:481–503.
3. Kelly AOJ. The patogenesis of appendicitis. Phil Med J 1899; 21:119–137.
4. Yildirim S, Nursal TZ, Tarim A, Kayaselcuk F, Noyan T. A rare cause of acute appendicitis: parasitic infection. Scand J Inf Dis 2005; 37:757–759.
5. Singh I, Collins RE. Right iliac fossa pain. Br J Hosp Med 2005; 66:76–78.
6. Karatepe O, Adas G, Tukenmez M, Battal M, Altiok M, Karahan S. Parasitic infestation as cause of acute appendicitis. G Chir 2009; 30:426–428.
7. Dorfman S, Cardozo J, Dorfman D, Del Villar A. The role of parasites in acute appendicitis of pediatric patients. Invest Clin 2003; 44:337–340.
8. Aydin Ö. Incidental parasitic infestations in surgically removed appendices: a retrospective analysis. Diag Pathol 2007; 2:16. http://www.diagnosticpathology.org/content/2/1/16.

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KARAPANDZIC FLAP FOR RECONSTRUCTION OF LOWER LIP IN A 18-MONTH OLD BOY WITH CONGENITAL AGAMMAGLOBULINEMIA AND ECTHYMA GANGRENOSUM. CASE REPORT

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Abstract. *The reconstruction of lower lip in children is extremely rare and challenging procedure. The etiology in literature reveals trauma and infection. An 18-month boy was admitted with sepsis and pneumonia. Laboratory tests revealed congenital agammaglobulinemia. Necrosis of lower lip developed and was diagnosed as ecthyma gangrenosum. Blood culture was positive for Pseudomonas aeruginosa. Multiple abscess formations were found in abdominal wall and gluteal region and were treated by incisions. After spontaneous demarcation of necrotic tissue in lower lip the Karapandzic flap technique was used for reconstruction. Karapandzic flap can be used as optimal method for reconstruction of lower lip in children with satisfactory functional and aesthetic results.*

Key words: *Karapandzic flap, ecthyma gangrenosum, reconstruction*

Introduction

The congenital agammaglobulinemia is inherited disease characterized by depletion of humoral immune response. The diagnosis is confirmed by low concentrations of blood immunoglobulins (IgG or IgM). In any case of immunocompromised pediatric patient, including aplastic anemia, leukemia or agammaglobulinemia, the high susceptibility for infections is recognized, mainly in the first four years of life. The key treatment is intravenous administration of immunoglobulins which boosts immune system [1].

One of the most common skin infections in immunocompromised children is ecthyma gangrenosum (EG). The EG can be a manifestation of any immunocompromised disease, such as hematological malignancies, severe burns, chemotherapy or immunodeficiency syndromes, including congenital agammaglobulinemia. Ecthyma gangrenosum is commonly associated with Pseudomonas aeruginosa bacteremia. It is almost always a sign of Pseudomonas sepsis. The clinical manifestation is recognized as hemorrhagic pustules or infracted skin appearing area with surrounding erythema. EG was first described in 1897 by Barker. Histology reveals necrotizing vasculitis. The final appearance is necrotic skin plaque surrounded by erythema [2, 3]. The lesion is mostly located in gluteal and perineal regions (57%), extremities 30%, trunk 6% and face 6% [3, 4]. The antibiotic therapy is the first treatment for initial lesion and must be defined according to blood culture sensitivity. Necrotic lesions must be treated by surgical debridement [5]. The most challenging are the locations of EG in the facial area where esthetic and functional requirements must be fulfilled [5, 6].

In case of lower lip defect, different reconstructive options must be taken into consideration. The goals are both aesthetic result and functional competence of lower lip (speech and food intake). The lip reconstruction can be performed using local flaps or microvascular free tissue transfer. One of the local flaps was described by Karapandzic and this flap was defined as myoneurovascular pedicled advancement flap [7]. The full-thickness lip defect requires reconstruction of all layers (mucosa, muscle, skin) and every case should be approached separately. The tissue from the cheek and the rest of the lower lip used for Karapandzic reconstruction most closely resembles the missing part of lower lip according to texture and color. Musculocutaneous flaps from both sides of the defect have sufficient height to restore the height of the lower lip and motor nerves are spared for appropriate functioning of orbicularis oris muscle. This flap is good for single stage lower lip reconstruction but it has significant limitations in microstomia [8].

Our aim was to present the young patient with ecthyma gangrenosum treated with Karapandzic flap technique.

Case Report

An 18-month boy was admitted in pediatric clinic with sepsis and bilateral pneumonia. Laboratory tests revealed

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congenital agammaglobulinemia. During the hospital stay, he developed multiple subcutaneous abscesses in the abdominal and gluteal region, as well as an abscess and necrotizing lesion in the central part of the lower lip diagnosed as ecthyma gangrenosum.

Blood culture was positive for pseudomonas aeruginosa and the antibiotics were administered according to blood culture sensitivity (Ceftazidime and Gentamycin). The immunoglobulins were administered. After 10 days, the patient's general health condition was stable. Initial surgical treatment included multiple incisions of abscesses and removal of necrosis from left groin region. The vasculitis in lower lip lead to necrosis of the central part of the lower lip. After spontaneous demarcation, initial scaring occurred (Fig. 1).

The defect in lower lip was reconstructed under general anesthesia using the Karapandzic flap technique. The postoperative result is shown in Figure 2.

Discussion

Ecthyma gangrenosum is a skin infection caused mostly by pseudomonas and manifested as skin necrosis surrounded by erythema [9–11]. It occurs in 1% to 30% of cases of Pseudomonas sepsis [12]. Other described etiology includes Klebsiella oxytoca or Escherichia coli and in this case an early broad spectrum antibiotic regimen is recommended for initial treatment of ecthyma gangrenosum [13, 14]. Lesions can occur anywhere but are more common in the perineum, buttocks, axillae, and extremities, but in our case they appeared in the central part of lower lip [15]. In some cases of ecthyma gangrenosum even facial nerve palsy with lesion in external auditory canal developed [16]. Surgical repair was planned when the lesion became stable [15].

In order to avoid excision of healthy tissue, the lower lip was not treated before spontaneous demarcation. The reconstruction of full thickness of the lower lip is a challenging procedure especially in children. The ecthyma gangrenosum occurs in the facial region in only 6 % of all cases. Spontaneous demarcation occurred after two weeks, leaving a full-thickness defect in the central part of the lower lip. Why did we perform the Karapandzic technique? In our opinion, it is the most acceptable technique for children because the myocutaneous flaps had the same skin color and texture as the missing part. The other reason is preservation of motor nerves. The operative procedure takes short time and surgery fulfills aesthetic requirements.

Some authors support the use of the Karapandzic flap, in its original form or modified, but some prefer labiomental flap, double rectangular rotation flap, the Fujimori gate flap from nasolabial area or staircase (step) technique [17–24]. Despite the fact that poor prognosis is associated with multiple lesions, as well as delayed treatment and neutropenia, our case had acceptable recovery [25]. Any reconstruction of the lips must include both functional and cosmetic considerations [19].

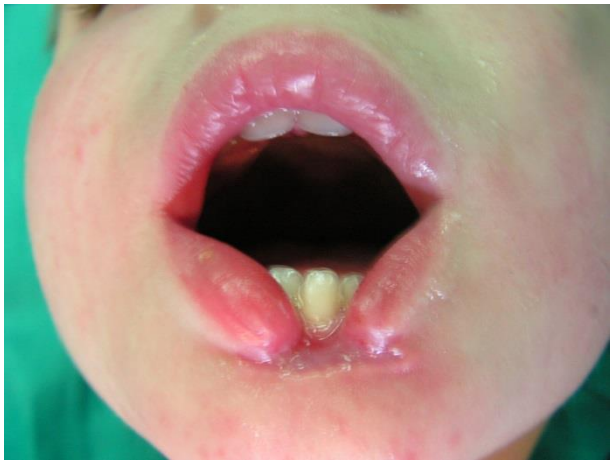

Fig. 1. Preoperative central lip defect

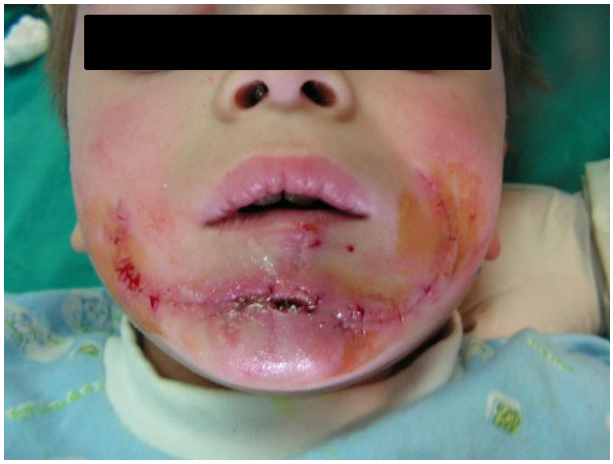

Fig. 2. Postoperative result after Karapandzic flap technique.

Conclusion

Satisfactory esthetic and functional results were obtained in a child with congenital agammaglobulinemia and ecthyma gangrenosum using Karapandzic flap technique for central part of the lower lip.

In our knowledge, this is the youngest patient treated with the Karapandzic flap technique.

References

1. Azar AE, Ballas ZK. Evaluation of the adult with suspected immunodeficiency. Am J Med 2007; 120:764–768.
2. Morimoto Y, Routes JM. Immunodeficiency overwiew. Prim Care 2008; 35:159–173.
3. Inamadar AC, Palit A, Athanikar SB, Sampagavi VV, Deshmukh NS. Periocular Ecthyma gangrenosum in a diabetic patient. Br J Dermatol 2003; 148: 821.
4. Ghosheh FR, Kakhuria SS. Bilateral periorbital ecthyma gangraenosum. Ophthal Plast Reconstr Surg 2006; 22:492-493.

5. Craigie RJ, Ahmed S, Mullassery D, Panarese A, Caswell M, Kenny SE. A spot that can kill. Lancet 2007; 369:1540.
6. Khalil BA, Baillie CT, Kenny SE, et al. Surgical strategies in the management of ecthyma gangrenosum in pediatric oncology patients. Pediatr Surg Int 2008; 24:793–797.
7. Karapandzic M. Reconstruction of lip defects by local arterial flaps. Br J Plast Surg 1974; 27:93–97.
8. Shohet MR, Khosh MM. Lip reconstruction. In: Buchen D (ed) Skin flaps in facial reconstruction. McGraw-Hill Co: New York, 2007; pp 127–147.
9. Koo SH, Lee JH, Shin H, Lee JI. Ecthyma gangrenosum in a previously healthy infant. Arch Plast Surg. 2012; 39:673–675.
10. Boisseau AM, Sarlangue J, Perel Y, Hehunstre JP, Taïeb A, Maleville J. Perineal ecthyma gangrenosum in infancy and early childhood: septicemic and nonsepticemic forms. J Am Acad Dermatol 1992; 27:415–418.
11. Biddeci G, Cutrone M, Mattei I, Valerio E, Favot F. Ecthyma gangrenosum of the cheek in a 6-month-old infant. Arch Dis Child 2014 http://adc.bmj.com/content/early/2014/07/22/archdischild-2014-306852.full.pdf+html
12. Zomorrodi A, Wald ER. Ecthyma gangrenosum: considerations in a previously healthy child. Pediatr Infect Dis J 2002; 21:1161–1164.
13. Prindaville B, Nopper AJ, Lawrence H, Horii KA. Chronic granulomatous disease presenting with ecthyma gangrenosum in a neonate. J Am Acad Dermatol 2014; 71:e44–45.
14. Pathak A, Singh P, Yadav Y, Dhaneria M. Ecthyma gangrenosum in a neonate: not always pseudomonas. BMJ Case Rep 2013. http://casereports.bmj.com/content/2013/bcr-2013-009287.full.pdf+html
15. Mota-Burgos A, Villa AV, Noguera-Julian A, Fortuny C, González-Enseñat MA. Fever and skin lesions in a healthy 6-month-old boy. Diagnosis: ecthyma gangrenosum. Pediatr Infect Dis J 2012; 31:789–794.
16. Gioacchini FM, Baccarani A, Villari D, Postacchini V, Alicandri-Ciufelli M. Ecthyma gangrenosum in a newborn causing external otitis with complete facial nerve palsy. J Cutan Med Surg 2014; 18:210–213.
17. Hanasono MM, Langstein HN. Extended Karapandzic flaps for near-total and total lower lip defects. Plast Reconstr Surg 2011; 127:1199–1205.
18. Sood A, Paik A, Lee E. Lower lip reconstruction: Karapandzic flap. Eplasty 2013; 13: ic17.
19. Aldelaimi TN, Khalil AA. Lip reconstruction using Karapandzic flap. J Craniofac Surg 2014; 25:e136–138.
20. Ye W, Hu J, Zhu H, Zhang C, Zhang Z. Application of modified Karapandzic flaps in large lower lip defect reconstruction. J Oral Maxillofac Surg 2014; 72:2077–2082.
21. Berridge N, Magopoulos C, Heliotis M. Bilateral labiomental fan flap: an alternative technique for one-stage total lower lip reconstruction. Plast Reconstr Surg 2014; 134:117.
22. Miyazaki H, Makiguchi T, Takayama Y, Yokoo S. Lower lip repair using double opposing rectangular rotation flaps with reconstruction of the mentolabial groove and mental protuberance. Int J Oral Maxillofac Surg 2014; 43:1073–1075.
23. Tetik G, Unlü E, Aksu I. Functional reconstruction of the lower lip with Fujimori flap and long-term follow-up with clinical and electrophysiologic evaluations. J Oral Maxillofac Surg 2014; 72:1841–1851.
24. Wollina U. Reconstruction of medial lower lip defects after tumour surgery: modified staircase technique. J Cutan Aesthet Surg 2013; 6:214-216.
25. Huminer D, Siegman-Igra Y, Morduchowicz G, Pitlik SD. Ecthyma gangrenosum without bacteremia. Report of six cases and review of the literature. Arch Intern Med 1987; 147:299–301.

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A GUIDE FOR THE PRACTICAL EXAM IN HUMAN ANATOMY

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edited and illustrated by Rade Čukuranović

– Critiques and Reviews –

Excerpts from Reviews

..All anatomical preparations come from human cadaveric material processed and prepared observing the most rigorous anatomical principles, demonstrating very clearly all the structures within respective regions, being equal in that regard with similar publications in the international literature. The organs also come from cadaveric material, being processed anatomically in a way that the details intended are clearly visible... The approach, processing procedures, and photographs are unique in our region and, I believe, in nearby countries as well, since there is no similar publication for practical instruction in anatomy, and it will be a useful supplemental tool to be used by the students of medicine and other related disciplines for exam preparation. The manuscript represents a collected practical experience during the years of hard work at the Department of Anatomy in Niš, being of great help in all clinical branches in which anatomy has a pivotal role...

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Faculty of Sport and Physical Education
University of Niš, Serbia

...This original manuscript, intended for practical exam preparation and attendance of practical classes in human anatomy, entirely fulfills its purpose. Illustrations are the main characteristic of the text, which is certainly most important in learning anatomy. Numerous photographs, all of them in color, are of very high quality, clear, and informative, supplementing adequately very ample learning contents presented to medical students. The contents are presented in an appropriate way, combining systematic and topographical approaches to morphology...

Prof. Dr. Slobodan Malobabić
Faculty of Medicine
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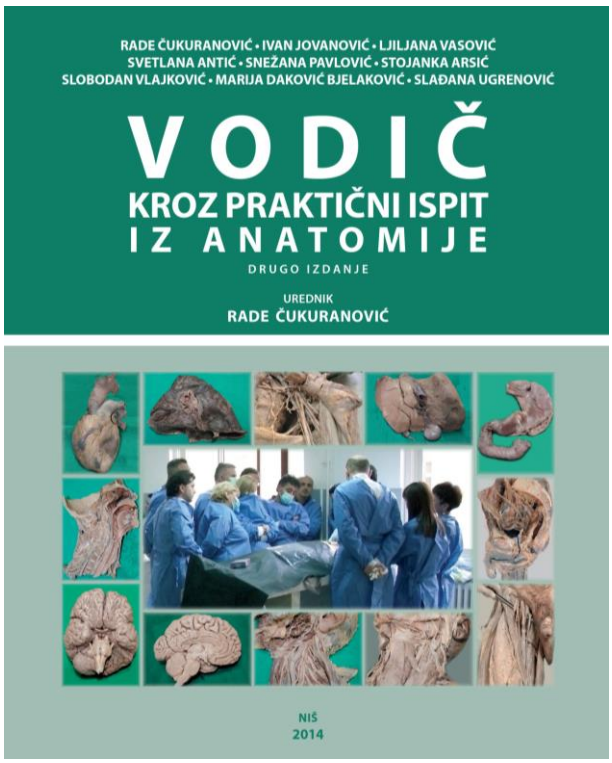

...An additional quality of the manuscript is contained in very well prepared and selected preparations, presenting, as a rule, different aspects of the same anatomic structure. Explanations of the illustrations are precise and sufficient, and preparations are marked in a clear and systematic way. I should also point out the precision in marking the necessary structures, feeling of appropriateness in the selection of preparations for practical exam, clear and very well done brain sections...

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