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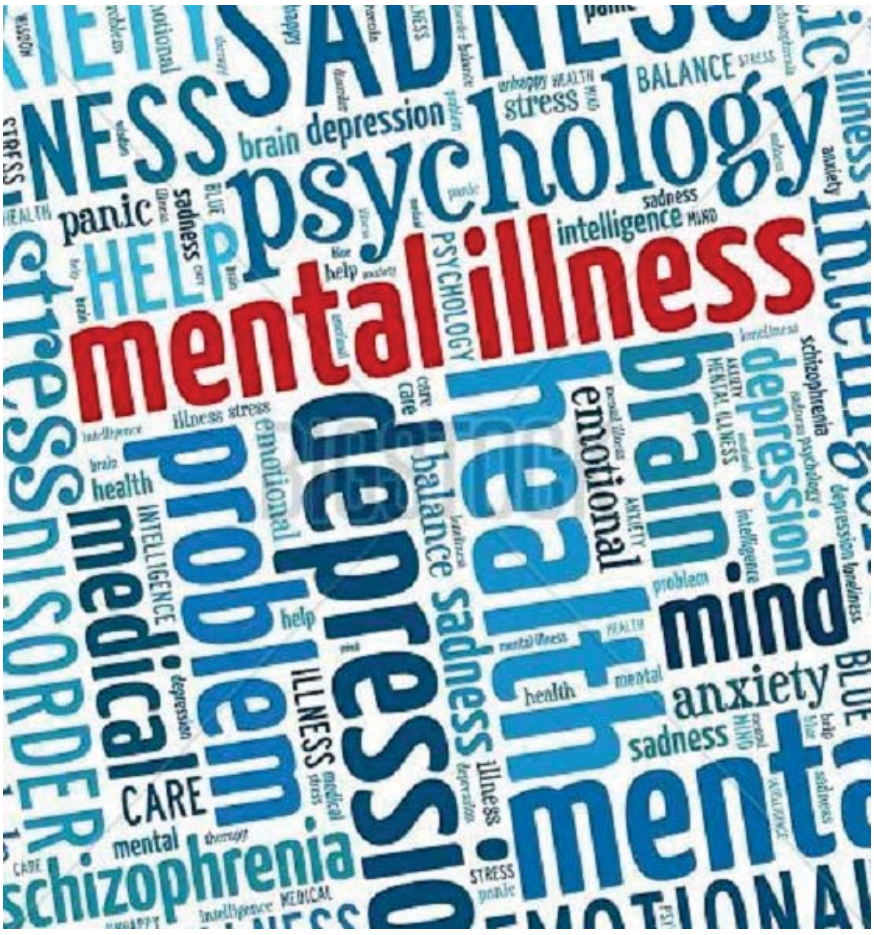

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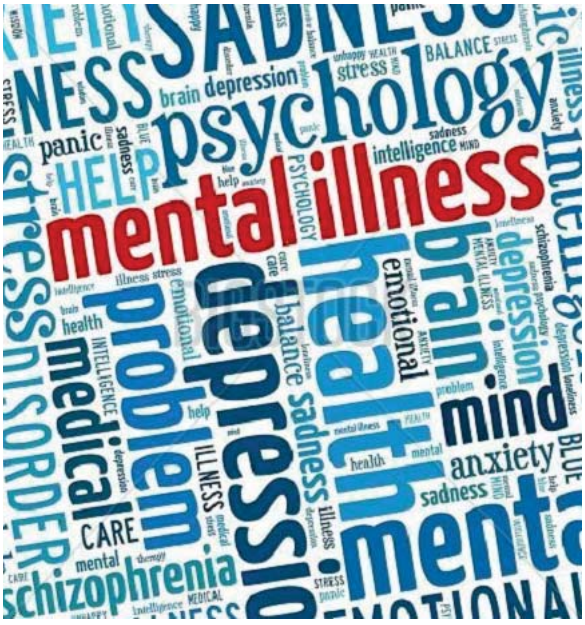

World Mental Health Day is marked on 10 October every year, with the overall objective of raising awareness of mental health issues around the world and mobilizing efforts in support of mental health.
The Day provides an opportunity for all stakeholders working on mental health issues to talk about their work, and what more needs to be done to improve mental health care quality for people worldwide.
The theme of the Day for 2014 is "Living with schizophrenia" that is in accordance with the effort of the World Health Organization to provide living a healthy life to patients with schizophrenia.

Svetski dan mentalnog zdravlja obeležava se svake godine 10. oktobra sa ciljem da se podigne svest o problemima mentalnog zdravlja u svetu i mobilisanja napora u podršci očuvanja mentalnog zdravlja.
Ovaj dan prilika je da svi zainteresirani koji rade na problemima mentalnog zdravlja govore o svom radu, kao i o tome što sve treba učiniti za poboljšanje kvaliteta zaštite mentalnog zdravlja celokupnog svetskog stanovništva.
Tema ovogodišnjeg Svetskog dana mentalnog zdravlja je „Živeti sa shizofrenijom", što je u skladu sa naporima Svetske zdravstvene organizacije da se obolelima od shizofrenije obezbedi zdrav života.

ORIGINAL ARTICLES

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The impact of diabetes mellitus on the course and outcome of pregnancy during a 5-year follow-up

Uticaj dijabetesa melitusa na tok i ishod trudnoće u 5-godišnjem praćenju

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Abstract

Background/Aim. Women with diabetes, especially diabetes type 1, have worse pregnancy outcomes, as well as increased incidence of spontaneous abortions, pre-eclampsia, fetal macrosomia, preterm delivery, congenital anomalies and perinatal mortality. The aim of this study was to analyze the course and outcome of pregnancy in the patients with diabetes in relation to the group of healthy women regarding preterm delivery, perinatal morbidity and mortality. Also, the aim was to compare pregnancy outcomes in the patients with pre-existing diabetes type 1 and the patients with gestational and diabetes type 2. **Methods.** This retrospective study included 156 diabetic women treated at the Clinic of Endocrinology, Diabetes and Metabolic Diseases and Gynecology and Obstetrics Clinic of the Clinical Center of Vojvodina from 2006 to 2010. There were 94 patients with gestational diabetes, 48 with type 1 diabetes, and 14 patients with type 2 diabetes. The control group included 106 healthy women hospitalized at the Gynecology and Obstetrics Clinic. **Results.** The women with type 1 diabetes presented with a statistically significantly higher incidence of cesarean section than those without diabetes, or with type 2 or gestational diabetes ($p < 0.0001$); the women with type 1 diabetes delivered at an earlier week of gestation (WG) in regard to women without diabetes, or with type 2 or gestational diabetes ($p = 0.0017$ and $p = 0.02$, respectively). The incidence of perinatal morbidity: hypoglycemia ($p < 0.001$), pathological jaundice ($p = 0.0021$), and other neonatal pathologies at birth ($p = 0.0031$), was statistically significantly higher and Apgar scores after 1 minute ($p = 0.0142$) and after 5 minutes ($p = 0.0003$) were statistically significantly lower in the patients with diabetes compared to the healthy women. The women with type 2 and gestational diabetes were statistically significantly older than those with type 1 diabetes ($p = 0.001$). A higher incidence of fetal macrosomia in the women with gestational and type 2 diabetes compared to those with type 1 diabetes was at the borderline of statistical significance ($p = 0.07$), whereas the incidence of hypoglycemia of newborn was statistically significantly higher in the patients with type 1 diabetes ($p < 0.0001$). Glycosylated hemoglobin (HbA1c) levels were statistically significantly higher in the diabetic women giving birth during and before the week of gestation 36 ($p = 0.0087$), but there were no differences in HbA1c levels in regard to fetal macrosomia ($p = 0.45$) and congenital abnormalities ($p = 0.32$). **Conclusion.** The results of our study show a higher incidence of perinatal fetal morbidity (hypoglycemia, jaundice, respiratory distress syndrome) in the patients with type 1, type 2 and gestation diabetes than in the healthy controls. Also, we found a higher incidence of cesarean section in the patients with type 1 diabetes than in those with type 2, gestation diabetes and healthy controls. Although delivery in the patients with type 1, type 2 and gestational diabetes was completed approximately one to two weeks earlier compared to the healthy controls there was no statistically significant difference in the incidence of preterm delivery (≤ 36th week of gestation) between the women with diabetes and healthy controls. Preterm delivery associated with poorer glycaemic control reflected through higher values of HbA1c in third trimester. Risks from adverse pregnancy outcomes may be reduced to minimum by adequate preconception counseling of diabetic patients and early diagnosis of diabetes in pregnancy, in order to achieve glycemic control during organogenesis and within pregnancy and through the teamwork of endocrinologists, gynecologists and pediatricians.

Key words:
diabetes mellitus; pregnancy; fetal development; obstetric labor, premature; morbidity.

Apstrakt

Uvod/Cilj. Žene sa dijabetesom, a posebno one sa tipom 1, imaju lošiji ishod trudnoće u odnosu na žene bez dijabetesa, pre svega zbog veće učestalosti spontanih pobačaja, preekalmpsije, makrozomije ploda, prevremenog porođaja, kongenitalnih malformacija i perinatalnog mortaliteta ploda. Cilj ispitivanja bio je analiza toka i ishoda trudnoće kod bolesnica sa dijabetesom u odnosu na kontrolnu grupu zdravih žena, a u odnosu na prevremeni porođaj, perinatalni

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morbiditet i mortalitet, kao i ishod trudnoće kod bolesnica sa preegzistentnim dijabetesom tipa 1 u odnosu na bolesnice sa gestacijskim i dijabetesom tipa 2. **Metode.** Retrospektivno istraživanje sprovedeno je na 156 žena sa dijabetesom lečenih na Klinici za endokrinologiju, dijabetes i bolesti metabolizma i Klinici za ginekologiju i akušerstvo Kliničkog centra Vojvodine tokom perioda 2006–2010. godine. Gestacijski dijabetes imalo je 94 ispitanice, dijabetes tip 1 48, a dijabetes tip 2 14 ispitanica. Kontrolnu grupu činilo je 106 zdravih žena hospitalizovanih u Klinici za ginekologiju i akušerstvo tokom 2011. i 2012. godine. **Rezultati.** Kod trudnica sa dijabetesom tipa 1 porođaj se statistički značajno češće završavao carskim rezom u odnosu na trudnice bez dijabetesa i trudnice sa dijabetesom tipa 2 i gestacijskim dijabetesom ($p < 0,0001$), kao i ranijoj nedelji gestacije u odnosu na trudnice bez dijabetesa i trudnice sa tipom 2 i gestacijskim dijabetesom ($p < 0,0017$, $p = 0,02$). Kod trudnica sa dijabetesom statistički značajno veća bila je učestalost hipoglikemije ($p < 0,0001$), patološkog ikterusa ($p = 0,0021$) i druge patologije ploda na rođenju ($p = 0,0031$) u odnosu na kontrolnu grupu zdravih trudnica uz lošije vrednosti Apgar scora u 1. minuti kod novorođenčadi ($p = 0,0142$) i 5. minutu ($p = 0,0003$). Trudnice sa dijabetesom tipa 2 i gestacijskim dijabetesom bile su statistički značajno starije nego trudnice sa dijabetesom tipa 1 ($p = 0,001$). Veća učestalost makrozomije ploda kod trudnica sa gestacijskim i dijabetesom tipa 2 u odnosu na tip 1 bila je na granici statističke značajanosti ($p = 0,07$), dok su hipoglikemije bile statistički značajno češće u grupi trudnica sa dijabetesom tipa 1 ($p < 0,0001$). Vrednosti glikozilovanog hemoglobina (HbA1c) bile su statistički značajno više kod trudnica sa dijabetesom porođenih tokom i pre 36. gestacijske nedelje ($p = 0,0087$), bez razlike u vrednostima HbA1c u odnosu na makrozomiju ploda ($p = 0,45$) i kongenitalne malformacije ($p = 0,32$). **Zaključak.** Rezultati našeg ispitivanja pokazuju višu učestalost perinatalnog fetalnog morbiditeta (hipoglikemije, ikterusa, respiratornog distres sindroma) kod bolesnica sa tipom 1, tipom 2 i gestacijskim dijabetesom u odnosu na kontrolnu grupu zdravih trudnica. Takođe, kod trudnica sa dijabetesom tipa 1 porođaj se češće završavao carskim rezom nego kod trudnica sa dijabetesom tipa 2 i gestacijskim dijabetesom i zdravih trudnica. Iako se trudnoća kod trudnica sa dijabetesom tipa 1, tipa 2 i gestacijskim dijabetesom završavala jednu do dve nedelje ranije nego kod zdravih trudnica, nije bilo statistički značajne razlike u učestalosti prevremenog porođaja (≤ 36. nedelje gestacije) između žena sa dijabetesom i zdravih trudnica. Prevremeni porođaj bio je povezan sa lošijom glikemijskom kontrolom iskazanom kroz više vrednosti glikoziliranog hemoglobina u trećem trimestru. Rizik od neželjenih ishoda trudnoće može se redukovati adekvatnim prekoncepcijskim savetovanjem bolesnica sa dijabetesom i pravovremenom dijagnozom dijabetesa u trudnoći, uz imperativ postizanja gotovo normoglikemijskog stanja kako u periodu organogeneze, tako i tokom cele trudnoće uz timski rad endokrinologa, ginekologa i pedijatra.

Ključne reči:
diabetes melitus; trudnoća; trudnoća, razvoj fetusa; porođaj, prevremeni; morbiditet.

Introduction

Impaired glucose metabolism is among the most common pregnancy-associated metabolic disorders occurring in 3–10% of all pregnancies. Gestational diabetes accounts for 88% of cases of diabetes in pregnancy, pre-existing diabetes type 2 accounts for 8%, and pre-existing type 1 for 4% [1, 2]. Epidemiological studies show that the prevalence of diabetes among reproductive-age women is increasing, probably due to insufficient physical activity, inadequate diet, and a great number of obese children and adolescents. That is why today more attention is paid to the problem of pre-existing diabetes type 2 in pregnancy and increasing incidence of gestational diabetes [3–5]. Up to the discovery and use of insulin, only a small number of pregnancies in diabetic women have been reported. In the 80's of the last century, risks of diabetes-related complications during pregnancy were 50% higher than in healthy pregnant women [2]. Although current insulin therapy has improved the quality of glycemic control, women with diabetes, especially those with type 1 diabetes compared with women without diabetes, have worse pregnancy outcomes, as well as increased incidence of spontaneous abortions, pre-eclampsia, fetal macrosomia, preterm delivery, congenital anomalies and perinatal mortality [5–8]. Numerous studies have shown that the incidence of adverse pregnancy outcomes in women with type 2 diabetes is the same as in women with type 1 diabetes, and worse than in the population of healthy women [3, 6, 7]. Gestational diabetes is defined as any degree of glucose intolerance with onset or first recognition during pregnancy [1, 2]. The most important risk factors for gestational diabetes include sedentary lifestyle, unbalanced diet, poor physical activity, obesity, family history of diabetes, and previous history of macrosomia. That is why oral glucose tolerance test (OGTT) is recommended to all pregnant women at risk for gestational diabetes. Women are recommended to take a 2-hour OGTT with 75 grams of glucose between their 24th and 28th week of pregnancy, and those at high risk of establishing pregnancy as well [1, 4, 5]. For diagnosis of gestational diabetes it is sufficient to detect only one pathological value during 2 hour OGTT: blood glucose ≥ 5.1 mmol/L at the start, ≥ 10.0 mmol/L after first hour and ≥ 8.5 mmol/L after second hour of the test. In diagnosis of pre-existing diabetes mellitus (type 1 and type 2) a few criteria are in the use: fasting blood glucose ≥ 7.0 mmol/L, postprandial blood glucose ≥ 11.1 mmol/L and/or glycosylated hemoglobin (HbA1c) ≥ 6.5% [1].

Many studies showed that adverse pregnancy outcomes (congenital anomalies, spontaneous abortions and perinatal mortality) were associated with poor glycemic control in the early pregnancy [6–8]. The critical period is before 7 weeks of gestation, that is during organogenesis. Preconception counseling is of utmost importance aiming at good glycemic control during preconception and in the early weeks of gestation (WG). According to the recommendations of the American Diabetes Association, target glycemic control parameters are clearly defined: fasting blood glucose < 5.3 mmol/L; 1 h

postprandial blood glucose < 7.8 mmol/L; 2h postprandial blood glucose < 6.7 mmol/L; HbA1c between 6.0% and 6.5% [1].

There are clearly defined protocols and recommendations for monitoring diabetic pregnant women, depending on the gestational age of pregnancy [2, 5, 9]. In the first trimester it is recommended to attend control of an endocrinologist and gynecologist once a month, to control HbA1c value every 4–6 weeks, regular day-night blood glucose profile control (glycemia daily inspection before each meal and at bedtime, and also 2–3 times weekly 1–2 hours after each meal and during the night), control of TSH, free T4 (before conception and in the first trimester), control of urea, creatinine, 24 hour proteinuria, complete blood count, urine, arterial tension and cardiovascular status, monitoring body weight, and ophthamological examination.

In the second, and especially in the third trimester controls become more frequent. Monitoring of glycemic profile becomes daily and control of glycosuria, acetonuria and HbA1c, as well as control of endocrinologist is recommended firstly every four weeks and later every two weeks. Control of urea, creatinine, 24 hour proteinuria, hepatogram (in suspected preeclampsia), urine, arterial tension, cardiovascular status and weight control is recommended at each visit to the doctor. Ophthalmological examination is obliged in the week of gestation 28, and in the presence of retinopathy in the period between the week of gestation 16 and 20.

Pregnant women with diabetes should be offered ultrasound monitoring of fetal growth and amniotic fluid volume every 4 weeks from 28 to 36 weeks. Women with diabetes and macrovascular disease and/or nephropathy will require an individualised approach to monitoring fetal growth and wellbeing [2, 5].

Diabetes mellitus is not an indication for cesarean section unless there are some other obstetric indications (fetal macrosomia, feto-pelvic disproportion, etc.) or maternal complications (advanced chronic complications of diabetes, eclampsia). But, evidence shows that women with diabetes are more likely to undergo induction of labour and/or caesarean section at 38–39 week of gestation than women without diabetes to prevent stillbirth and shoulder dystocia, which are associated with fetal macrosomia [5].

Elective birth should be offered after completed the week of gestation 38 in the cases of a good metabolic control of diabetes, absence of abnormalities of the fetus, respiratory distress syndrome and other obstetric and maternal complications. Preterm delivery is indicated in cases of poor metabolic control of diabetes, fetal growth acceleration or intrauterine fetal growth retardation, fetal respiratory distress syndrome, polyhydramnios or other obstetric and/or maternal complications [3, 5].

The aim of this study was to analyze the components of maternal and perinatal morbidity in pregnant women with diabetes in relation to the group of healthy pregnant women and separately among the groups with pre-existing type 1 diabetes and gestational and type 2 diabetes, as well as to assess a correlation of components of metarnal and perinatal outcomes in regard to glycemic control, duration of diabetes and chronic complications.

Methods

This retrospective study included 156 diabetic women in the week of gestation 28–32 treated at the Clinic of Endocrinology, Diabetes and Metabolic Diseases and Gynecology and Obstetrics Clinic of the Clinical Center of Vojvodina, Novi Sad, from 2006 to 2010. There were 94 patients with gestational diabetes, 48 with type 1 diabetes, and 14 with type 2 diabetes. Considering a small number of women with type 2 diabetes diagnosed with diabetes mellitus before pregnancy and treated only with diet and/or metformin therapy, bearing in mind similar etiopathogenetic mechanism as gestational diabetes, we included them in the same group for statistical analysis. The control group included 106 healthy women also in the week of gestation 28–32 giving birth at the Gynecology and Obstetrics Clinic of the Clinical Center of Vojvodina during 2011 and 2012. The control group was randomly selected among healthy pregnant women hospitalized in 2011 and 2012 considering the fact that socioeconomic living conditions, health care system and management of pregnancy were not changed in relation to the period 2006–2010. The following parameters were studied: maternal age, arterial hypertension (blood pressure over 140/90 mmHg or use of antihypertensive therapy), and preeclampsia during pregnancy (the diagnosis of pre-eclampsia was made after the week 20 of gestation in cases with a arterial hypertensia and proteinuria > 300 mg/24 h), type of delivery (vaginal or cesarean), gestational age at delivery, and the number of spontaneous abortions in previous pregnancies. Neonatal characteristics included Apgar scores, birth weight, birth length, hypoglycemia, jaundice or any other perinatal morbidity and perinatal mortality. These groups were also examined for differences in HbA1c levels, whereas in patients with diabetes type 1 types of insulin therapy and incidence of microvascular complications of diabetes were investigated as well. Statistical data processing was carried out by *t*-test and test of proportion.

Results

Table 1 shows perinatal and maternal outcomes among the pregnant women with type 1 diabetes and the healthy control. The incidence of delivery by cesarean section was statistically significantly higher compared to the non-diabetic women ($p < 0.0001$), and the women with type 1 diabetes were more likely to deliver earlier than women without diabetes (37.9 weeks of gestation *vs* 39.09 weeks of gestation, respectively, $p < 0.0017$). In addition, perinatal morbidity was statistically significantly higher in the diabetic women compared to the healthy controls: hypoglycemia ($p < 0.001$), pathological jaundice ($p = 0.0021$), and other causes of neonatal morbidity ($p = 0.0031$). Accordingly, Apgar scores after 1 minute ($p = 0.0142$) and after 5 minutes ($p = 0.0003$) were statistically significantly lower in the diabetic than in non-diabetic women. There were no differences in birth

Table 1

Perinatal and maternal outcomes observed among the pregnant women with type 1 diabetes and the healthy controls

Parameters	Patients with type 1 diabetes	Healthy controls	*p*
Age (years), $\bar{x} \pm SD$	28.83 ± 5.13	30.57 ± 5.15	0.0537
Arterial hypertension (%)	8.51	10.38	0.9450
Pre-eclampsia (%)	2.13	6.60	0.4443
Spontaneous abortions in previous pregnancies (%)	25.40	14.15	0.1422
Gestational age at delivery (weeks), $\bar{x} \pm SD$	37.90 ± 1.55	39.09 ± 2.36	0.0017
Delivery ≤ 36 WG (%)	14.58	9.43	0.5047
Cesarean section (%)	78.26	35.85	< 0.0001
Apgar score I, $\bar{x} \pm SD$	7.91 ± 1.33	8.57 ± 1.61	0.0142
Apgar score II, $\bar{x} \pm SD$	8.78 ± 0.97	9.39 ± 0.93	0.0003
Birth weight (g), $\bar{x} \pm SD$	3463.62 ± 576.57	3396.60 ± 697.28	0.5617
Birth length (cm), $\bar{x} \pm SD$	50.32 ± 2.41	49.70 ± 3.24	0.2380
Fetal hypoglycemia (%)	52.17	1.88	< 0.0001
Fetal jaundice (%)	50.00	23.58	0.0021
Other fetal morbidity at birth (%)	68.75	41.51	0.0031
Congenital malformations (%)	6.83	13.21	0.3755
Fetal macrosomia (%)	10.42	16.98	0.4157
Stillbirth (%)	4.20	0.00	0.1743

weight and birth length of newborns between the two examined groups. However, in regard to birth weight, one must bear in mind that pregnancies in the women with diabetes completed on the average 1–2 weeks earlier than in the women without diabetes, so it may be assumed that the newborns of the women with diabetes would have had a statistically significantly higher birth weight if they had been born in the same week of gestation as the newborns of the healthy controls. There were no statistically significant differences between these two groups of women in relation to the incidence of congenital abnormalities, spontaneous abortions in previous pregnancies and stillbirths.

Table 2 shows characteristics of the patients with type 1 diabetes related to perinatal fetal morbidity. The women whose infants exhibited perinatal morbidity (hypoglycemia, jaundice, diabetic fetopathy, respiratory distress syndrome) had longer duration of diabetes, higher incidence of chronic complications, lower glucose control, but these differences were not statistically significant. In addition, there were no statistically significant differences regarding the incidence of cesarean section and lower gestational age at birth, birth weight and birth length. A statistically significant difference was only found in Apgar score value in the 1 minute (p = 0,036).

Table 3 shows perinatal and maternal outcomes observed among the pregnant women with type 1 diabetes compared with women with type 2 diabetes and gestational diabetes.

The patients with type 2 diabetes and gestational diabetes were statistically significantly older than the patients with type 1 diabetes ($p = 0.001$). The glycemic control was better in the patients with type 2 diabetes and gestational diabetes than in the patients with type 1 diabetes ($p < 0.0001$). The incidence of hypertension and pre-eclampsia was higher in the patients with type 2 diabetes and gestational diabetes, but the difference was not statistically significant. The incidence of cesarean section ($p = 0.0001$) and completion of pregnancy 1–2 weeks before full-term ($p = 0.0093$) were statistically signifi-

Table 2

Characteristics of the patients with type 1 diabetes related to perinatal fetal morbidity

Parameters	Without fetal morbidity	With fetal morbidity	*p*
Age (years), $\bar{x} \pm SD$	29.93 ± 4.73	28.36 ± 5.29	0.3426
Duration of diabetes (years), $\bar{x} \pm SD$	8.00 ± 5.71	11.91 ± 6.86	0.0608
Insulin analogue therapy (%)	33.33	45.45	0.9470
Microvascular diabetic complications (%)	13.33	25.81	0.5614
HbA_1c (%), $\bar{x} \pm SD$	6.48 ± 0.90	7.10 ± 1.14	0.1139
Arterial hypertension (%)	6.67	9.37	0.8014
Pre-eclampsia (%)	6.67	0.00	0.6945
Spontaneous abortions in previous pregnancies (%)	26.67	24.24	0.8577
Gestational age at birth (weeks), $\bar{x} \pm SD$	38.09 ± 0.81	37.81 ± 1.81	0.5712
Cesarean section (%)	85.71	75.00	0.6731
Apgar score I, $\bar{x} \pm SD$	8.47 ± 0.99	7.61 ± 1.38	0.0367
Apgar score II, $\bar{x} \pm SD$	9.00 ± 0.53	8.68 ± 1.11	0.2970
Birth weight (g), $\bar{x} \pm SD$	3362.67 ± 386.97	3510.94 ± 646.85	0.4172
Birth length (cm), $\bar{x} \pm SD$	50.07 ± 1.83	50.44 ± 2.66	0.6292

Table 3

Perinatal and maternal outcomes observed among the pregnant women with type 1 diabetes compared with the women with type 2 and gestational diabetes

Parameters	Patients with type 1 diabetes	Patients with type 2 diabetes and gestational diabetes	*p*
Age (years), $\bar{x} \pm SD$	28.83 ± 5.13	31.89 ± 5.32	0.0010
HbA_1c (%), $\bar{x} \pm SD$	6.85 ± 1.14	5.85 ± 0.79	< 0.0001
Arterial hypertension (%)	8.51	15.32	0.3701
Pre-eclampsia (%)	2.13	4.54	0.7895
Spontaneous abortions in previous pregnancies (%)	25.40	17.86	0.3816
Gestational age at delivery (weeks), $\bar{x} \pm SD$	37.90 ± 1.55	38.50 ± 1.19	0.0093
Delivery ≤ 36 WG (%)	14.58	5.35	0.1004
Cesarean section (%)	78.26	41.28	0.0001
Apgar score I, $\bar{x} \pm SD$	7.91 ± 1.33	8.10 ± 1.16	0.3760
Apgar score II, $\bar{x} \pm SD$	8.78 ± 0.97	8.93 ± 0.91	0.3616
Birth weight (g), $\bar{x} \pm SD$	3463.62 ± 576.57	3570.72 ± 579.48	0.2892
Birth length (cm), $\bar{x} \pm SD$	50.32 ± 2.41	50.58 ± 2.18	0.5077
Fetal hypoglycemia (%)	52.17	16.51	< 0.0001
Fetal jaundice (%)	50.00	49.54	0.9017
Other fetal morbidity at birth (%)	68.75	45.54	0.0117
Congenital malformations (%)	6.83	7.14	0.7896
Fetal macrosomia (%)	10.42	24.11	0.0770
Stillbirth (%)	4.20	0.00	0.1721

cantly higher in the patients with type 1 diabetes than in those with type 2 and gestational diabetes. Perinatal morbidity was statistically significantly higher in the patients with type 1 diabetes, especially hypoglycemia ($p < 0.0001$). There were no statistically significant differences in relation to birth weight, birth length, and Apgar scores of newborns between the patients with type 1 diabetes and those with type 2 diabetes and gestational diabetes. The incidence of fetal macrosomia was higher in the patients with gestational and type 2 diabetes (24.11%) than in those with type 1 diabetes (10.4%), that is considered as borderline statistical significance. The incidence of preterm delivery was higher in the patients with type 1 diabetes (14.58%) in relation to the patients with gestational and type 2 diabetes (5.35%), but the difference was not statistically significant. In addition, there was no statistically significant difference in the incidence of congenital abnormalities and spontaneous abortions in previous pregnancies between the patients with diabetes type 1 and those with diabetes type 2 and gestational diabetes.

Table 4 shows perinatal and maternal outcomes observed among the pregnant women with type 2 diabetes and gestational diabetes in relation to the healthy controls.

There were no statistically significant age related differences between the patients with diabetes and healthy controls. There were also no statistically significant differences in the incidence of hypertension, pre-eclampsia and cesarean section between these two groups. In the patients with type 2 diabetes and gestational diabetes, pregnancies ended at the

Table 4

Perinatal and maternal outcomes observed among the pregnant women with type 2 diabetes and gestational diabetes in relation to the healthy controls

Parameters	Patients with type 2 and gestational diabetes	Healthy controls	*p*
Age (years), $\bar{x} \pm SD$	31.89 ± 5.32	30.57 ± 5.15	0.0643
Arterial hypertension (%)	15.32	10.38	0.3762
Pre-eclampsia (%)	4.54	6.60	0.7126
Cesarean section (%)	41.28	35.85	0.4941
Spontaneous abortions in previous pregnancies (%)	17.86	14.15	0.5747
Delivery ≤ 36 WG (%)	5.35	9.43	0.3708
Gestational age at delivery (weeks), $\bar{x} \pm SD$	38.50 ± 1.19	39.09 ± 2.36	0.0197
Apgar score I, $\bar{x} \pm SD$	8.10 ± 1.16	8.57 ± 1.61	0.0138
Apgar score II, $\bar{x} \pm SD$	8.93 ± 0.91	9.39 ± 0.93	0.0003
Birth weight (g), $\bar{x} \pm SD$	3570.72 ± 579.48	3396.60 ± 697.28	0.0457
Birth length (cm), $\bar{x} \pm SD$	50.58 ± 2.18	49.70 ± 3.24	0.0190
Fetal hypoglycemia (%)	16.51	1.88	0.0005
Fetal jaundice (%)	49.54	23.58	0.0001
Other fetal morbidity at birth (%)	45.54	41.51	0.6433
Congenital malformations (%)	7.14	13.21	0.2069
Fetal macrosomia (%)	24.11	16.98	0.2574
Stillbirth (%)	0.00	0.00	–

week of geastation 38.5, that was statistically significantly earlier compared to the control group – the week of gestation 39.09 ($p = 0.02$). However, Table 4 also shows that the patients with diabetes exhibited a statistically significantly higher incidence of hypoglycemia ($p = 0.0005$), pathological jaundice ($p = 0.0001$), birth weight ($p = 0.0457$), and birth length ($p = 0.019$), first minute Apgar score ($p = 0.0138$), and 5 minute Apgar score ($p = 0.0003$), compared to healthy controls. Nevertheless, we should keep in mind that pregnant women with diabetes completed their pregnancies on the average one week earlier than the healthy controls, which probably affected lower incidence of fetal macrosomia.

Table 5 shows HbA1c values in relation to birth weight, week of gestation at delivery and congenital anomalies among the pregnent women with diabetes.

Table 5

HbA_1c as a parameter of glycemic control, among the pregnant women with diabetes (type 1, type 2 and gestational) in relation to birth weight, week of gestation (WG) at delivery and congenital anomalies

Parameters	HbA_1c (%), $\bar{x} \pm SD$	*p*
Birth weight > 4000 g	6.04 ± 0.88	0.4515
Birth weight < 4000 g	6.26 ± 1.07	
Delivery ≤ 36 WG	6.78 ± 1.31	0.0087
Delivery > 36 WG	6.18 ± 1.00	
With congenital anomalies	6.55 ± 1.47	0.3255
Without congenital anomalies	6.19 ± 1.01	

HbA_1c (%) – glycosylated hemoglobin

The women giving birth before the week of gestation 36 presented with a statistically significantly higher levels of HbA1c in relation to those completing their pregnancies after the week of gestation 36 ($p = 0.0087$), but there were no statistically significant differences in the levels of HbA1c in relation to macrosomia and congenital anomalies.

Discussion

In recent decades, due to current insulin therapy, monitoring of pregnant women with diabetes, counseling, preconception care and strict glucose control, the incidence of perinatal mortality and congenital abnormalities showed a significant reduction in diabetic patients [9–12]. However, despite improvements in glycemic control, the incidence of fetal macrosomia is still 20–40%. Fetal macrosomia is usually defined as birth weight over 95% [13, 14]. Macrosomia is recognized as a cause of fetal morbidity and mortality and despite relatively good glycemic control, its incidence is statistically higher in mothers with diabetes in relation to non-diabetic mothers. Macrosomia also increases maternal morbidity, frequently requiring instrumental delivery or cesarean section. It seems that accelerated fetal growth is determined by HbA1c levels in the first half of pregnancy and it continues despite improved glycemic control in the second half of pregnancy.

Maternal hyperglycemia leads to fetal hyperglycemia and consequently to fetal pancreatic beta-cell hyperplasia. Poon et al. [15] investigated 33,602 women and found that infant birth weight was associated not only with pre-existing and/or gestational diabetes, but also correlated with maternal weight and height, and that it was higher in multipara in regard to nulipara. In our study, there were no statistically significant differences in infant birth weights of mothers with type 1 diabetes and those of healthy controls. This is partly a consequence of the fact that pregnancies in women with type 1 diabetes are completed on the average one or two weeks earlier compared to healthy controls. Our results show that the incidence of neonatal macrosomia was highest in patients with type 2 diabetes and gestational diabetes (24.11%) compared to patients with type 1 diabetes (10.5%) and healthy controls (16.98%), but these differences were not statistically significant. These results can be explained by the fact that on the average glycemic control in our diabetic patients (HbA1c of 6.5%) was satisfactory, and it is well-known that fetal macrosomia is mostly associated with high HbA1c levels in the second and third trimesters of pregnancy. This is certainly contributed by the fact that delivery in patients with diabetes type 1 is usually planned between the week of gestation 36 and 38 and particularly in those with obstetrically verified accelerated fetal growth. A higher incidence of fetal macrosomia in patients with type 2 diabetes and gestational diabetes than in patients with type 1 diabetes may be because fetal macrosomia is affected not only by diabetes, but also by maternal obesity, which is more often associated with type 2 diabetes and gestational diabetes.

During labor and birth process, infants of mothers with diabetes are at increased risk for neonatal hypoglycemia, due to interrupted glucose supply and hyperinsulinism [2, 16]. Perinatal stress is also associated with neonatal hypoglycemia, in part because of catecholamine and glycocorticoid-stimulated mobilization and depletion of glycogen stores. Reactive hypoglycemia occurs within 2 hours after childbirth and persists up to 72 hours, but may last up to one week. Up to 50% of infants of mothers with type 1 diabetes experience hypoglycemia after birth. The results of our study are in accordance with these results showing that the incidence of neonatal hypoglycemia was 52% in mothers with type 1 diabetes, and 16.5% in mothers with type 2 diabetes or gestational diabetes. Both groups of diabetic mothers presented with a statistically significantly higher incidence of neonatal hypoglycemia compared to healthy controls without recorded hypoglycemia. Hrabovski et al. [16] also established a higher incidence of hypoglycemia, hypocalcemia and hypomagnesemia in infants of diabetic mothers compared to healthy controls.

The incidence of hypoglycemia is higher in macrosomic infants, but also in infants with low birth weight in relation to

infants with normal birth weight. Neonatal hypoglycemia in infants small-for-date is the consequence of intrauterine growth retardation, often found in mothers with long-term diabetes and chronic vascular complications [2, 17, 18]. Newborn infants of diabetic mothers are also at increased risk of hyperbilirubinemia, which is explained by increase in the red blood cell count and mass, ineffective erythropoiesis and relative immaturity of hepatic conjugation and bilirubin excretion [2, 17, 18]. Both groups of diabetic women (type 1 and type 2 or gestational diabetes) presented with 50% of pathological neonatal jaundice, which is statistically significant in regard to healthy controls. These results are in agreement with the results of numerous previous studies, which confirmed the occurrence of pathological jaundice in neonates of diabetic women [17, 18].

The perinatal mortality is about five times higher in women with diabetes than in those without diabetes. Apart from carbohydrate metabolism disorders, diabetes is also associated with metabolic disorders involving fats, proteins, and amino acids, all of which inevitably affects fetal gene expression and increases teratogenicity. Risks of fetal death are certainly associated with maternal obesity, hypertension, advanced age and long-term diabetic complications [19, 20]. In our study, stillbirths were recorded only in patients with type 1 diabetes (4.2%), whereas the percentage of stillbirths in previous pregnancies was 1.7%, that was not statistically significant in relation to healthy controls and patients with type 2 diabetes and gestational diabetes, where the percentage of stillbirths in previous pregnancies was 0.9% and there were no recorded stillbirths in actual pregnancies. Spontaneous abortion is the best indicator of glycemic control in the preconception period and the early weeks of pregnancy. Investigations have documented that HbA1c levels above 11% increase risks of early spontaneous abortions by over 40% [21, 22] Although the incidence of spontaneous abortions was higher in patients with type 1 diabetes compared to patients with gestational, and type 2 diabetes, and healthy controls, the difference was not statistically significant. The incidence of congenital malformations is 3–5 times higher in women with pre-existing diabetes than in healthy controls, and there is no difference between patients with type 1 and type 2 diabetes. Congenital anomalies often cause stillbirth, especially cardiovascular anomalies and neural tube defects. However, according to the same data, maternal diabetes alone is not a predictive factor of specific anomalies [2, 21]. Although literature data mostly suggest that the incidence of congenital anomalies is higher in women with diabetes, our study showed no statistically significant differences in relation to the healthy controls [21, 22]. HbA1c levels were higher in women with fetal congenital abnormalities in regard to those without them, but the difference was not statistically significant. Major congenital malformations are most commonly associated with hypeglycemia in the preconception period and during organogenesis, but some authors reported about certain nervous system malformations associated with hypoglycemia in the first trimester. Preterm births are closely associated with perinatal mortality and morbidity. One third of preterm births are among mothers with pregestational diabetes. It is believed that the percentage of preterm deliveries (spontaneous and induced) in women with diabetes type 1 is about 45%. The most important predictive factors for preterm delivery include poor glycemic control and vascular complications (pre-eclampsia and nephropathy) [23]. In our study, there was statistically significantly poorer glycemic control in the women which gave birth before the week of gestation 36, and that matches with literature data [23]. Type 1 diabetic patients had more frequent preterm delivery and the most of the cases occurred between the week of gestation 34–36, in regard to patients with type 2 and gestational diabetes and healthy subjects, but without a statistically significant difference. The Confidential Enquiry into Maternal and Child Health (CEMACH) has also reported that the preterm delivery rate is 5 times higher in patients with type 1 and type 2 diabetes compared to general population [5]. About 2/3 of preterm deliveries are medically induced, most often due to fetal complications. The occurrence of hypertension in patients with diabetes type 1 is commonly the consequence of the onset and/or progression of diabetic nephropathy, whereas patients with diabetes type 2 mostly present with essential hypertension, as part of the cardiometabolic syndrome. Both groups of patients are at higher risk for developing pre-eclampsia, especially patients with pre-existing diabetic nephropathy [23, 24]. The above-mentioned maternal vascular complications have been consistently associated with pre-term delivery, perinatal fetal morbidity and mortality [25]. In patients with type 1, type 2 and gestational diabetes, the incidence of hypertension and pre-eclampsia was not statistically significantly different compared to healthy controls. The incidence of hypertension and pre-eclampsia was slightly higher in patients with gestational and type 2 diabetes in relation to patients with diabetes type 1, but the difference was not statistically significant. These results can be explained by the fact that the majority of patients with type 1 diabetes, compared to those with type 2 diabetes or gestational diabetes, get pregnant at a younger age, with a shorter duration of diabetes, without diabetic nephropathy or in its incipient phase. Patients with type 1 diabetes and perinatal morbidity presented with longer duration of diabetes, higher incidence of microvascular complications (macrovascular complications were not diagnosed), hypertension and higher HbA1c levels. The above-mentioned differences were not statistically significant, maybe due to a small number of patients with type 1 diabetes included in the study.

Conclusion

The results of our study show a higher incidence of perinatal fetal morbidity (hypoglycemia, jaundice, respiratory distress syndrome) in the patients with type 1, type 2 and gestation diabetes than in the healthy control. Also, we found a higher incidence of cesarean section in the patients with type 1 diabetes than in those with type 2 diabetes, gestation diabetes and healthy control. Although delivery in the patients with type 1, type 2 and gestational diabetes was completed approximately one to two weeks earlier compared

to the healthy control group there was no statistically significant difference in the incidence of preterm delivery (before the week of gestation 36) between the women with diabetes and the healthy control group. Preterm delivery associated with poorer glycaemic control reflected through higher values HbA1c in third trimester. Bearing in mind that the mentioned complications are usually associated with hyperglycemia, it is necessary to provide adequate preconception counseling of diabetic patients, timely diagnosis of diabetes, and good glycemic control both during organogenesis and throughout pregnancy, in order to reduce adverse pregnancy outcomes to minimum.

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Condition of periodontium in patients with fixed orthodontic appliances

Stanje periodoncijuma kod pacijenata sa fiksnim ortodontskim aparatima

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Abstract

Background/Aim. Orthodontic patients should be familiar with techniques of maintaining oral hygiene as well as with proper methods of checking maintenance of oral hygiene. The aim of this study was to determine a correlation between condition of periodontium and techniques of maintaining oral hygiene in patients treated with fixed orthodontic appliances. **Methods.** The research population included 100 patients, aged 15–25, treated by the orthodontist from 2005 to 2010. The maintenance of oral hygiene and the condition of periodontium was assessed using the following indices: plaque index, gingival index, bleeding index and oral hygiene index. The study was carried out using data obtained from the especially designed questionnaire as well as by objective examination of periodontal condition in accordance with the World Health Organization methodology, using adequate indicators and indices. **Results.** The results of the study show a significant correlation between condition of periodontium and oral hygiene in those with fixed orthodontic appliances. The use of interdental brushes and mouthwash liquid, as well as teeth brushing, were among the most significant predictors of healthy teeth and mouth. **Conclusion.** Teeth and mouth hygiene determined by frequency of teeth brushing, using of interdental brushes and mouthwash liquid are the basic preconditions for preservation and promotion of tooth and mouth health in patients with fixed orthodontic appliances.

Key words:
orthodontic appliances; periodontium; oral hygiene; dental plaque index; periodontal index; questionnaires.

Apstrakt

Uvod/Cilj. Ortodonski pacijent mora da poznaje tehnike održavanja higijene usta i zuba kao i metode za pravilnu provjeru održavanja higijene usta i zuba. Cilj rada bio je da se utvrdi korelacija između stanja zdravlja zuba i faktora sredine, odnosno navika kod ispitivanih osoba koje izvjestan vremenski period nose fiksnu protezu. **Metode.** Istraživanje je obavljeno na 100 ortodonskih pacijenata, starosti od 15 do 25 godina, koji su posjećivali ordinaciju ortodonta u periodu 2005–2010. godine. Vršena je procjena zdravlja usta i zuba na osnovu sledećih indeksa: plak indeksa, gingivalnog indeksa, indeksa krvarenja i indeksa oralne higijene. Istraživanje je rađeno na osnovu podataka dobijenih iz posebno napravljenog upitnika i na osnovu objektivnog istraživanja zdravlja usta i zuba, po usvojenoj metodologiji Svetske zdravstvene organizacije, a na osnovu adekvatnih indikatora. **Rezultati.** Utvrđena je statistički značajna povezanost između stanja zdravlja usta i zuba i održavanja oralne higijene kod osoba sa fiksnim ortodonskim aparatima. Kao značajni prediktori zdravlja usta i zuba izdvojili su se korišćenje interdentalne četkice i tečnosti za ispiranje usta, kao i redovno pranje zuba. **Zaključak.** Higijena usta i zuba, koju određuju učestalost pranja zuba i korišćenje interdentalnih četkica i tečnosti za ispiranje usta i zuba, osnovni su preduslovi očuvanja i unapređenja zdravlja usta i zuba osoba za fiksnim ortodontskim aparatom.

Ključne reči:
ortodontski aparati; periodoncijum; usta, higijena; zub, indeks plaka; periodontalni indeks; upitnici.

Introduction

A correct position of teeth in the dental arch as well as a proper relationship between the jaws, are both essential for proper functioning of the orofacial system. This also provides the most favourable decomposition of forces released during mastication and uniform load distribution on teeth and joints. Most people have some degree of malocclusion, but disturbances in function and aesthetics do not require treatment for all of them [1]. Adequate orthodontic treatment is required in cases with the position of teeth causing overloading of jaw joint and individual teeth, as well as more rapid tooth decay due to development of periodontitis and periodontal disease. In addition, incorrect tooth position may also allow retention of food debris and bacteria which can stimulate dental plaque formation and its impact on the development

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of caries and periodontal disease. Aesthetic reason is only the third among the most important reasons for orthodontic treatment, although it is almost the sole reason for patients to visit a dentist. If there is any of the abovementioned reasons for orthodontic treatment it is necessary to ensure integrated activities of a patient, an orthodontist and dentists of other specialties [2]. All of them play a role and have responsibility as complete for adequate mouth and tooth hygiene in orthodontic patients, which is one of the preconditions of oral health. Plaque formation during orthodontic treatment may cause hyperplastic gingivitis with increased pocket depth. The tendency of elevated plaque formation was observed in cases of increase in basic changes on the surface of the enamel. Therefore, the highest standards of oral hygiene should be applied during the orthodontic treatment [3].

Orthodontic patients should be familiar with techniques of maintaining mouth and tooth hygiene, as well as with proper methods of checking mouth and tooth hygiene. All their carious teeth should also be treated so that endodontic treatment of teeth could be done. For orthodontic patients, especially those with fixed appliances, regular check-ups are very important in order to prevent functional and aesthetic improvement at the expense of increased carious activity and gingival tissues diseases [4]. It is a well-known fact that orthodontic patients have problems with a large number of pathogenic microorganisms because wearing an appliance easily increases accumulation of groups of bacteria. Because of that, it is necessary to provide them with adequate information and knowledge regarding proper and complete oral and dental hygiene. It is the duty of orthodontists to involve the patient in a systematic program for prevention of dental caries and periodontal disease with activities directed towards removal of pathogenic microorganisms. In this way possible harmful pathological effect of orthodontic treatment can be prevented [5].

The aim of this study was to determine a correlation between condition of periodontium and techniques of maintaining oral hygiene in patients wearing fixed appliances for a certain period of time.

Methods

The research was conducted on 100 patients, aged 14–26 treated at the Dental Clinic of the Tivat Medical Center by the orthodontist from 2005 to 2010. They were asked to take part in the research and the decision was theirs. For those who were minors their parents were asked and they decided on their behalf. Examinations were conducted during regular dental check-ups of patients with fixed orthodontic appliances (braces). The patients were in good general health and did not receive fluoro-prophylactic treatment. A dentist mirror and a probe were used in accordance with World Health Organisation criteria. The assessment of oral hygiene index (OHI) was carried out in all the examined patients. It included the assessment of soft layers in the following way: 0 indicated that no soft layers on teeth; 1 indicated soft layers covering 1/3 of the tooth surface; 2 indicated soft layers covering 2/3 of the tooth surface.

In order to assess the condition of periodontium the following indices were used [6]: plaque index (PI), gingival index (GI) and gingival bleading index (GBI).

PI refers to examination and probing while determining the presence and quantity of dental plaque in gingival third of vestibular and oral side of each tooth and its numerical marking [6]: 0 point indicates that dental plaque cannot be detected neither with examination nor with probing; 1 point – dental plaque cannot be seen but it can be detected by probing; 2 points – moderate amount of dental plaque that can be detected by both examination and probing; 3 points – a large amount of dental plaque can be easily seen.

PI was calculated by dividing the sum of the obtained values, first by the number of the examined teeth and then by the number of the examined sides.

GI determines the presence and the degree of gingival inflammation. Examination was performed to determine whether there was inflammation [6]. If it was present, the degree of inflammation was numerically graded on the vestibular and oral side of each tooth: 0 – inflammation is not present; 1 – mild inflammation, slight redness and/r mild gingival swelling; 2 – inflammation, redness and/or gingival swelling; 3 – severe inflammation, marked redness and/or gingival swelling.

GI was calculated by dividing the sum of the obtained values first by the number of examined teeth and then by number of examined sides.

GBI determines the presence and the extent of gingival bleeding and indirectly the degree of inflammation [6]. A periodontal probe was pulled through gingival sulcus from the vestibular side of each tooth in the first and third quadrant and from the oral side of each tooth in the second and fourth quadrant. The extent of bleeding was then numerically graded: 0 – no bleeding; 1 – spot bleeding; 2 – line bleeding; 3 – blood fills the entire gingival sulcus; 4 – blood spills out from the sulcus.

GBI was calculated by dividing the sum of the obtained values by the number of the examined teeth.

In addition to clinical examination the questionnaire was used as a research instrument. Data on sociodemographic characteristics of respondents as well as data on their habits related to oral health were obtained from the questionnaire.

Statistical analysis

Statistical analysis was performed by using Statistica version 6 (StatSoft Inc., Tulsa, SAD) statistical package. Each index (OHI, PI and GI) was calculated separately for the maxilla and the mandible for each patient by summing the scores for each sector and dividing the sum by three. Overall oral hygiene index (OOHI) was calculated by summing all the three indexes for each sector for the maxilla or the mandible and dividing the sum by six. Average oral indexes were calculated by summing each of four indexes for maxilla with each for mandible and dividing the sum by two. This was done so that the results for each of the presented index would be comparable with the initially used scale for assessment of the indexes (0–3). The results were presented

as arithmetic means and standard errors of the means (SE). All the variables were normalized using logarithmic transformation before the analysis of variance (ANOVA). The changes in hygiene indexes over time together with changes over time corresponding to different categories of several factors with a possible influence on the level of hygiene indexes (age, gender, type of fixed prosthodontic appliance, constructive material, placement of appliance in maxilla and/or mandible) were analysed using repeated measures ANOVA using only one factor for each analysis. Multifactorial analysis was not done because of the small sample that would result in uneven and incomplete design. When age was used as factor sample was divided into quartiles and age quartiles were used as levels. As gender did not show any significant difference in any of the analyses of hygiene indexes dynamics ($p > 0.20$ for all) these results were not shown. As the calculus index did not show variability at the time point of temporary luting for its analysis Friedman ANOVA was used. The value of $p < 0.05$ was considered as statistically significant for all analyses.

Results

The research included a total of 100 respondents, more than half of them were female, and their education level and occupation varied (Figure 1). The results show a statistically highly significant correlation between PI and the frequency of tooth brushing. Most of the respondents with no plaque brushed their teeth more than twice a day (Table 1).

The results also show a statistically significant correlation ($p < 0.001$) between GBI and the frequency of teeth brushing (Table 2). Gingival bleeding was statistically significantly less frequently diagnosed in patients who brushed their teeth more than twice a day, which was statistically significant. Grades 3 and 4 of the GBI were not found among the patients.

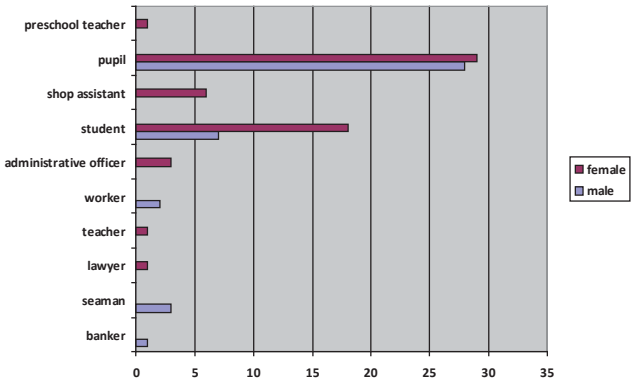

Fig. 1 – Distribution of the patients with fixed orthodontic appliances by sex and occupation.

Table 3 shows a statistically highly significant correlation between OHI and the frequency of tooth brushing. Higher values of OHI were found to be statistically significantly more frequent in the patients who brushed their teeth up to twice a day or less often. In those patients layers of soft tissues were identified on a larger surface of the teeth (Table 3).

Multiple logistic regression models were used to determine a correlation between PI and hygiene habits of respondents with assumed formation of two groups. Plaque was not diagnosed in one group but it was diagnosed in the other one.

The variables which were identified as predictors of risk for diagnosing PI were: use of interdental brushes and mouthwash liquid. Namely, those respondents who regularly used interdental brushes and mouthwash liquid were statistically significantly less frequently at risk of being identified plaque index. The same values were also presented in the risk of occurrence of GI (Table 4).

In the second model with a dependent variable that represents the risk level for occurrence of GBI, two separate

Table 1

Prevalence of plaque index (PI) values in patients with fixed orthodontic appliances in dependence of tooth brushing frequency

The clinical sing and PI point	PI		Total
	twice a day	> twice a day	
No plaque (0)	8	26	34
Thin layer of plaque (1)	38	4	42
Moderate amount of plaque (2)	24	0	24
Large amount of plaque (3)	0	0	0
Total	70	30	100
Pearson $\chi^2 = 53.635$; $p < 0.001$			

Table 2

Prevalence of gingival bleeding index (GBI) values in patients with fixed orthodontic appliances in dependence of tooth brushing frequency

The clinical sing and GBI point	Frequency of tooth brushing		Total
	twice a day	> twice a day	
No bleeding (0)	12	28	40
Spot bleeding (1)	38	0	38
Line bleeding (2)	22	0	22
Total	72	28	100
Pearson $\chi^2 = 51.005$; $p < 0.001$			

Table 3

Prevalence of oral hygiene index (OHI) values in patients with fixed orthodontic appliances in dependence of tooth brushing frequency

The clinical sing and OHI point	Frequency of tooth brushing		Total
	twice a day	> twice a day	
No soft layers (0)	13	28	41
Layers up to 1/3 of teeth (1)	37	4	41
Layers from 1/3 to 2/3 of teeth (2)	18	0	18
Total	69	34	100
Pearson $\chi^2 = 56.811$; $p < 0.001$			

Table 4

Variables – predictors of risk groups in the logistic model of plaque index

Risk predictors	B	S.E.	Wald	df	*p*	Exp(B)
Use of interdental brushes	3.897	1.152	11.447	1	0.001	49.246
Use of mouthwash liquid	2.287	0.986	5.379	1	0.020	9.844
Constant	-9.212	5.810	2.514	1	0.113	0.000

B – regression coefficient; S.E. – standard error; Wald – Wald statistical test which tests statistical significance of each coefficient (b); df – degrees of freedom; *p* – statistical significance; EXP(B) – relative risk , that is odd ratio.

levels were identified: no gingival bleeding and gingival bleeding. This model determined the following sequence of variables – risk predictors: the use of interdental brushes, the use of mouthwash liquid and tooth brushing (Table 5).

In the third logistic regression model dependent variable was the risk measured by OHI in relation to oral hygiene habits. This model singled out the use of interdental brush as a significant predictor of plaque formation (Table 6).

Clark [7] believes that the knowledge, skills and motivation are the basis of adequate oral hygiene. It is therefore essential that during any dental check-up dentists make comments on patient's oral hygiene, so that they can learn how to apply the knowledge about oral hygiene. Dentists should motivate them in order to get positive feedback [7]. The study results also highlight the importance of adequate and comprehensive information on the application of modern tech-

Table 5

Variables – risk group predictors in the logistic model of gingival bleeding

Risk predictors	B	S.E.	Wald	df	*p*	Exp(B)
Tooth brushing	-2.565	1.114	5.302	1	0.021	0.077
Use of interdental brush	3.578	1.153	9.629	1	0.002	35.790
Use of mouthwash liquid	3.354	1.262	7.064	1	0.008	28.620
Constant	-5.437	5.750	0.894	1	0.344	0.004

For explantation of abbriviations, see the note under Table 4.

Table 6

Variables – risk group predictors in the logistic model of OHI

Risk predictors	B	S.E.	Wald	df	Sig.	Exp(B)
Use of interdental brush	3.326	1.044	10.152	1	0.001	27.820
Constant	43.282	12969.702	0.000	1	0.997	6.26618

For explantation of abbreviations, see the note under Table 4.

Discussion

Adequate oral hygiene of patients undergoing orthodontic treatment with a fixed appliance implies the involvement of a specialist (an orthodontist), a general dentist, parents and a patient in the active treatment process. The study shows a statistically significant correlation between the use of modern means of oral hygiene and oral health indicators values. The frequency of teeth brushing significantly determines the condition of oral health measured by appropriate indicators. Patients with a fixed orthodontic appliance who brush their teeth more frequently than twice a day have statistically significantly more favourable values of appropriate indicators of oral health.

niques as well as training in their application in order to preserve and improve oral health.

The results of earlier rescarchs it can be suggest that the condition of oral health in the examined patients with fixed orthodontic appliance could be improved by taking measures such as health education, training and revising. Oral hygiene techniques should also be checked and constant motivation of patients should be provided [8, 9]. The similar results were confirmed by this study which found that only tooth brushing was not enough for the complete protection of oral health when the fixed orthodontic appliances were used. The importance of using interdental brushes and mouthwash liquid by all patients was also stressed especially by those with a fixed appliance.

Some authors suggest two major factors for orthodontis to take into account specific needs of patients who were the subjects of the examination (disease susceptibility, condition of the mouth) and individual characteristics (preferences, manual dexterity and lifestyle) [10]. However, there is no doubt that taking adequate and concrete measures and following the recommended procedures can significantly preserve oral health of patients who wear fixed orthodontic appliances.

It is very important to consider the efficiency and predictability of psychological and educational methods used to follow the guidelines for maintaining oral hygiene in orthodontic patients as well as the consequences of failure to comply with these guidelines. Consistent application of appropriate knowledge and skills can contribute to saving time and money and reduce the risk of unwanted treatments which are often associated with poor oral hygiene [9].

Conclusion

The study shows oral hygiene as significantly correlated with oral health of patients who underwent orthodontic treatment with fixed appliances. Frequency of tooth brushing correlates with the condition of oral health assessed by the relevant health indicators. Additionally, interdental brush and mouthwash liquid as measures of oral hygiene are significant variables of oral health in patients with fixed orthodontic appliances.

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Morphological and functional outcome of scleral buckling surgery compared to primary vitrectomy in patients with retinal detachment

Morfološki i funkcionalni ishod klasične hirurške metode u odnosu na *pars plana* vitrektomiju kod bolesnika sa ablacijom retine

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Abstract

Background/Aim. Among the proposed operative techniques for retinal detachment (RD) the most commonly applied are classical method with scleral buckling and *pars plana* vitrectomy (PPV). The aim of this paper was to determine which surgical intervention of these two leads to better morphological results in terms of the applied retina and better functional outcomes in terms of visual acuity (VA) of the operated eye in patients with RD. **Methods.** A retrospective study on the comparative section of the effects of scleral buckling surgery and PPV in uncomplicated rhegmatogenous RD was performed. In a 2-year period 97 patients, i.e. 98 eyes with RD were operated on (68 eyes with scleral buckling surgery *vs* 30 by PPV). **Results.** In the group with classically operated detachment, the retina was applied in 52 (76.5%) cases *vs* 30 (100%) patients in PPV group ($p < 0.05$). Postoperative VA in logMAR was significantly better in both groups compared to preoperative VA: in the classically operated was 1.89 ± 1.04 preoperatively *vs* 0.98 ± 0.70 postoperatively, while in the PPV group, preoperative value was 2.56 ± 0.67 *vs* 1.31 ± 0.74 postoperatively (p = 0.001). **Conclusion.** PPV in uncomplicated forms of RD gives better anatomical results than scleral buckling surgery. VA was significantly improved in both observed groups, while its mean value was postoperatively better in the group that was operated with the classical method. The reason for this could be due to better VA in baseline in the scleral buckling surgery group.

Key words:
retinal detachment; scleral buckling; vitrectomy; visual acuty; treatment outcome.

Apstrakt

Uvod/Cilj. Najčešće hirurške metode rešavanja ablacije retine su klasična metoda sa serklažom i *pars plana* vitrektomija (PPV). Cilj ovog rada bio je da se utvrdi koja od navedenih hirurških tehnika ima bolje morfološke (u smislu naleganja retine) i funkcionalne rezultate (vidna oštrina). **Metode.** Ova retrospektivna studija sprovedena je tokom dve godine na ukupno 98 očiju kod 97 bolesnika sa regmatogenom ablacijom retine koji su operisani ili klasičnom metodom sa serklažom (68 očiju) ili PPV metodom (30 očiju). **Rezultati.** U grupi bolesnika operisanih klasičnom metodom, retina je nalegla kod 52 (76,5%) oka *vs* 30 (100%) oka operisana PPV ($p < 0,05$). Postoperativna VA (u logMAR) bila je značajno bolja kod obe grupe u odnosu na preoperativne vrednosti: kod klasične metode preoperativna VA iznosila je1,89 ± 1,04 *vs* postoperativno 0,98 ± 0,70. U PPV grupi, preoperativna VA iznosila je 2,56 ± 0,67 *vs* 1.31 ± 0.74 postoperativno ($p = 0.001$). **Zaključak.** PPV kod nekomplikovanih regmatogenih ablacija retine daje bolje anatomske i morfološke rezultate nego klasična operacija sa serklažom. Vidna oštrina je postoperativno bila bolja u obe grupe, ali je srednja vrednost vidne oštrine bila bolja u grupi operisanoj klasičnom metodom, verovatno zato što je PPV grupa imala lošiju inicijalnu vidnu oštrinu.

Ključne reči:
retina, ablacija; vitrektomija; serklaž vitrektomija; vid, oštrina; lečenje, ishod.

Introduction

Despite the continual improvement of surgical techniques applied, retinal detachment (RD) remains an ocular condition that results in a loss or reduction of visual acuity (VA). Among the proposed operative techniques for RD, the most commonly applied are classical method with scleral buckling and *pars plana* vitrectomy (PPV).

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Classic surgery with scleral buckling is considered to be an effective method for solving uncomplicated rhegmatogenous retinal detachment. It is known, however, that this surgical method is a subject to a number of operative and postoperative complications such as: intrusion, extrusion and implant infection, ocular motility disorder, change of refraction, anterior segment ischemia, macular distortion and cystoid macular edema [1–4]. During external drainage of subretinal fluid the following may occur: subretinal hemorrhage, retinal incarceration or rupture of the retina.

Primary PPV is an alternative to classical surgery. A direct access to the vitreous traction using microsurgical instruments could produce better results with fewer complications. Results in the literature about possible advantages of a primary vitrectomy over the traditional surgery of retinal detachment are controversial. There are papers that report better anatomical and functional results of PPV compared to scleral buckling surgery [5–7], but a number of authors do not find advantages of PPV in uncomplicated rhegmatogenous retinal detachment over classical operation [8–11].

The aim of this paper was to determine which surgical intervention of these two has better morphological results in terms of the applied retina and better functional outcome in terms of VA of the operated eye in the patients with uncomplicated rhegmatogenous RD.

Methods

This retrospective study on the comparative section of the effects of scleral buckling surgery with PPV in uncomplicated rhegmatogenous retinal detachment was conducted at the University Eye Clinic"Prof. Dr. Ivan Stankovic" of the Clinical Hospital Center "Zvezdara". In a 2-year period (January 2010–December 2011) there were 97 patients, i.e. 98 eyes with RD operated on. The analysis did not include eyes with vitreous hemorrhage, proliferative vitreoretinopathy (PVR) in a more distinct stadium (stadium PVR $\geq$ C), with non-rhegmatogenous retinal detachment, traumatic detachment and previously operated eyes. All operations were performed under local peribulbar anesthesia by the some surgeon. In a classical technique 2 mm scleral buckling and 5.5 mm stopping were used. External drainage of subretinal fluid was performed in all cases, while a gas sulfur hexafluoride (SF6) was installed intravitreally when it was necessary. Primary three-port PPV was performed by releasing vitreous traction around the rupture and removing of the front vitreous by scleral indentation. An internal drainage of subretinal fluid was performed through a rupture or retinotomy as well as retinopeksy using endolaser photocoagulation. Tamponade was performed with silicone oil, for the cases with retinal rupture in three and more quadrants, and gas. The patients with gas installed were ordered positioning of the head in the first 24 hours after the surgery. All the patients underwent the same postoperative local therapy consisted of antibiotics, corticosteroids and cycloplegics for 1–2 months. Postoperative controls were carried out on a daily basis the first 7 days, then after 1, 3 and 6 months following the surgery. The following data were collected: age and sex of patients, right/left eye, lens status (phakia, aphakia, pseudophakia), refraction (emetropia, myopia/hyperopia < 5 and $\geq$ 5 dioptre), localization and number of retinal ruptures, undetected retinal ruptures, localization and size of retinal detachment, the presence of proliferative vitreoretinopathy, affected or unaffected macula, morphological outcome i.e. applied retina, best corrected visual acuity before and after the surgery. Visual acuity was presented from the Snellen chart to the LogMAR chart (logarithm of the minimal resolution angle) in a way that the mark 0 in the LogMAR chart is equivalent to the VA of 1.0 in the Snellen chart, while +1.0 is equivalent to the VA of 0.1 in the Snellen. Counting fingers before the eye corresponds to the value of +2.0 LogMAR, while hand moving in front of the eye is marked by + 3.0 LogMAR.

Data were analyzed by descriptive statistical methods and statistical tests (*t*-test, χ^2-square test, Fisher exact probability test, and Wilcox on matched- pairs test), with a *p* value equal or less than 0.05 (two-sided) considered to indicate statistical significance.

Results

During the observed 2-year period 98 eyes with retinal detachment were operated on. In 68 (69%) eyes retinal detachment was operated by the classic method and these cases were considered as the first group of examinees. In 30 (31%) eyes retinal detachment was operated with PPV method and these cases accounted for the second group of examinees. Clinical characteristics of the patients are shown in Table 1. Two groups of patients were not differentiated by the age and gender. The right eye was statistically more frequently surgically treated classic method than by PPV ($p < 0.05$). Clinical status of lens did not differ in the two groups. Namely, in the group with classical surgery there were 51 (75%) phakic eyes versus about 23 (76.6%) phakic eyes with PPV. There was no significant difference between the groups regarding lens status ($p = 0.90$). Preoperative refraction could be determined in only 47 (69.1%) eyes in the first group and in 16 (53%) eyes in the second group. Emetropia was demonstrated in 7 cases in the first group *vs* 4 cases in the PPV group, hyperopia in 14 cases in the first group *vs* 4 cases in the PPV group, myopia > -5 dioptre in 11 cases in the first group *vs* 4 cases in the PPV group, and myopia $\leq$ 5 dioptre in 15 casesin the first group *vs* 4 in the PPV group.

Regarding the presence of retinal rupture, 48 (83.33%) cases in the first group and 18 (72.2%) in the second group demonstrated it. In vitrectomised eyes giant ruptures were more represented than in the eyes operated on with classical method. The two groups did not differ statistically regarding the localization of ruptures ($p = 0.82$), but they differed regarding the involvement of the macula with detachment ($p = 0.05$). In the first group detachment mostly affected two quadrants of the retina in 28 (41%) eyes, while in the second group it was mostly registered that four quadrants of retina were affected by detachment, in 19 (63%) eyes.

Anatomical success differed statistically between the two groups (Table 2). In the group with classically operated

Table 1

Epidemiologic and clinical data about the patients with retinal detachment

Parameter	Scleral buckling surgery	*Pars plana* vitrectomy	*p*
Number of patients, n (%)	68 (69)	30 (31)	
Age of patients (years), $\bar{x} \pm SD$	56.9 ± 10.5	57.9 ± 16.9	0.78
Sex, n (%)			
male	39 (57)	20 (67)	0.52
women	29 (43)	10 (33)	
Eye with retinal detachment, n (%)			
right	48 (71)	14 (46)	0.04
left	20 (39)	16 (54)	
Lens status, n (%)			
phacic	51 (75)	23 (77)	0.90
aphakia/pseudophakia	17 (25)	7 (23)	
Refraction, n (%)			
unknown	21 (31)	14 (46)	
emetropia	7 (10)	4 (13.5)	
myopia < 5D	15 (22)	4 (13.5)	
myopia ≥ 5D	11 (16)	4 (13.5)	
hyperopia	14 (21)	4 (13.5)	
Retinal rupture, n (%)			
0	20 (29)	12 (40)	
1	40 (58)	13 (43)	
2	7 (10)	3 (10)	
≥ 3	1 (3)	2 (7)	
Retinal rupture localization, n (%)			
superior	33 (70)	12 (67)	
inferior	13 (28)	5 (28)	0.82
giant rupture	2 (2)	6 (5)	
Macular involvement, n (%)			
yes	34 (50)	22 (73)	
no	34 (50)	8 (27)	
Retinal detachment localization, n (%)			
superior	17 (25)	3 (10)	
inferior	19 (28)	4 (13)	
superior and inferior	32 (47)	23 (77)	
Retinal detachment size, n (%)			
1 quadrant	12 (18)	1 (3)	
2 quadrants	28 (41)	7 (24)	
3 quadrants	17 (25)	3 (10)	
4 quadrants	11 (17)	19 (63)	

Table 2

Morphologic and functional outcome of different surgery methods in the patients with retinal detachment

Outcome of retinal detachment surgery	Scleral buckling surgery (n = 68)	*Pars plana* vitrectomy (n = 30)	*p*
Morphologic outcome, n (%)			
retina attached	52 (76.5)	30 (100)	0.006
re-detached retina	16 (23.5)	0 (0)	
Functional outcome (log MAR), $\bar{x} \pm SD$			
preoperative VA	1.89 ± 1.04	2.56 ± 0.67	0.001
postoperative VA	0.98 ± 0.70	1.31 ± 0.74	0.04

VA – visual acuity.

detachment, the retina was applied in 52 (76.5%) cases, while in the other group with performed vitrectomy the retina was applied in all 30 (100%) of the patients ($p < 0.01$).

Compared groups regarding the preoperative and postoperative VA, the mean value of VA (expressed as log MAR), was significantly better postoperatively than preoperatively ($p = 0.0001$) as shown in Table 2. The mean VA was higher in the scleral buckling surgery group but, however, it should be noted that the initial VA was worse in the PPV group.

Discussion

The goal of retinal detachment surgery is closure of retinal ruptures and release of vitreous traction on the retina. Classic surgery leads to release of the radial vitreous traction and bringing the retina into closer contact with the retinal pigment epithelium. Most retinal detachments can be favorably solved with this surgical method. The exceptions are patients with posterior ruptures and giant ruptures of the retina as well as patients facing technical difficulties fraught with scleral buck-

ling placement (patients with thin sclera, with previous strabismus surgery, glaucoma patients with drainage implants, etc.). Favorable results are achieved also with PPV that enables instrumental release of vitreous traction, gas instillation and internal subretinal fluid drainage in order to achieve the attached retina at the operating table itself. After primary PPV tamponade by intraocular gas and head positioning are always performed [12]. It is known that PPV increases the risk of developing cataracts and increased intraocular pressure (IOP). For these reasons, PPV is traditionally considered the second therapeutic option for resolution of primary RD, especially in cases with inferior ruptures. The exceptions to this are RD with giant ruptures where PPV is recommended as the first surgical procedure or PPV combined with scleral buckling. However, as reported by a number of authors, PPV gives good results if applied as the first surgical method for solving retinal detachment, too [7, 13, 14]. This is especially valid for patients with pseudophakia [12, 13]. According to de la Rúa et al. [15], scleral buckling surgery increases the risk of PVR, especially in pseudophakic eyes. The study of Heimann et al. [16] shows a benefit of scleral buckling in phakic eyes with respect to BCVA improvement. For pseudophacic patients, from the point of anatomical outcome, Heiman et al. [16] recommend PPV. Also, in the second study, the same authors analyzed the influence of the surgeon on anatomical and functional outcome ant they state that there is a statistically significant correlation between the surgeon and functional success in phakic group who underwent RD surgery while anatomic outcomes in both subgroups of phakic and pseudophakic patients showed no statistically significant correlation between surgeon and anatomic success [17].

By retrospective analysis of our results we find that the functional outcome of operation of retinal detachment performed in two methods differs. Namely, VA was significantly improved in both groups observed, while its average value was postoperatively better in the group operated with the classical method. The reason for this result may lie in the fact that the average preoperative VA was better in the group of classically operated detachment. Achieved functional improvement agrees with the results of recent retrospective studies [4, 5, 18]. Thus, a better anatomical success was achieved with primary vitrectomy than with classic retinal detachment surgery. This result agrees with the announcement of Azad et al. [19] and differs from some studies that reported failure of primary vitrectomy in 8% to 20% cases [4, 5] The difference is probably attributable to the advanced PVR changes in their cases. One of the arguments in favor of primary vitrectomy for rhegmatogenous retinal detachment was also faster eyesight recovery [4]. The argument against it could be the development of cataracts as a complication of primary vitrectomy [4, 5].

Conclusion

Classical operation with scleral buckling is a surgical procedure suitable for solving primary uncomplicated rhegmatogenous retinal detachment with transparent optical media that allows good visualization of the ocular fundus. *Pars plana* vitrectomy as primary operation of uncomplicated forms of rhegmatogenous retinal detachment, gives good anatomical and functional results. Bearing in mind cataract development, however, and increased intraocular preasure in some cases as a complication, it is reserved for retinal detachment with giant ruptures, posterior ruptures, as well as for redetachments after scleral buckling surgery.

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Prognostic value of heart rate variability in post-infarction patients

Prognostički značaj varijabilnosti srčane frekvencije kod bolesnika nakon infarkta miokarda

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Abstract

Background/Aim. Depressed heart rate variability (HRV) indicating autonomic disequilibrium and propensity to ventricular ectopy can be useful for risk stratification in patients following acute myocardial infarction (AIM). The aim of the study was to assess heart rate variability as a predictor of all-cause mortality in post-infarction patients. **Methods.** We analyzed the 24-hour electrocardiographic (ECG) recordings of 100 patients (80 males) during hospitalization for AIM. The mean age of patients was 56.99 + 11.03 years. Time domain heart rate variability analysis was obtained from 8 to 13 days after index infarction by mean of a 24-hour ECG recording, and the calculated parameters were: standard deviation of all normal to normal RR intervals (SDNN), RRmax-RRmin (difference between the longest RR interval and the shortest RR interval), mean RR interval. We also analyzed ventricular premature complexes from the ECG data. The patients underwent clinical evaluation, laboratory tests and echocardiography. **Results.** Within a one-year follow-up period 11 patients experienced death, 10 of them because of cardiac reason and one because of stroke. There were significantly lower values of SDNN (60.55 ± 12.84 ms *vs* 98.38 ± 28.21 ms), RRmax-RRmin (454.36 ± 111.00 ms *vs* 600.99 ± 168.72 ms) and mean RR interval (695.82 ± 65.87 ms *vs* 840.07 ± 93.97 ms) in deceased patients than in the survivors, respectively ($p < 0.01$). The deceased patients were of higher mean age, with lower left ventricular ejection fraction (0.46 ± 0.05 *vs* 0.56 ± 0.06 in survivors), and more frequent clinical signs of heart failure and ventricular ectopic activity (> 10VPCs/h; $p < 0.01$). Multivariate Cox analysis showed that SDNN was a significant, independent predictor of all-cause mortality in post-infarction patients. The other independent predictors were clinical signs of heart failure – Killip class II and III and ventricular ectopic activity. **Conclusion.** Depressed HRV is an independent predictor of mortality in post-infarction patients and may provide useful additional prognostic information in non-invasive risk stratification of these patients.

Key words:
myocardial infarction; heart rate; arrhythmias, cardiac; mortality; risk factors; predictive value of tests.

Apstrakt

Uvod/Cilj. Smanjena varijabilnost srčane frekvencije kao pokazatelj neuravnoteženosti autonomnog nervnog sistema i sklonosti ka ventrikularnim ekstrasistolama može biti korisna za stratifikaciju rizika kod bolesnika nakon akutnog infarkta miokarda. Cilj studije bio je da se ispita prognostički značaj varijabilnosti srčane frekvencije za ukupni mortalitet kod ovih bolesnika. **Metode.** Analizirali smo 24-časovno Holter elektrokardiografsko praćenje 100 bolesnika za vreme hospitalizacije zbog akutnog infarkta miokarda. Prosečna starost bolesnika bila je 56,99 + 11,03 godine, od kojih je bilo 80 muškaraca. *Time domain* analiza varijabilnosti srčane frekvencije određivana je od 8. do 13. dana od nastanka akutnog infarkta miokarda, uz upotrebu 24-časovnog Holter posmatranja, a izračunavani parametri bili su: standardna devijacija svih normalnih RR intervala (SDNN), RRmax-RRmin (razlika između najdužeg RR intervala i najkraćeg RR intervala) i prosečni RR interval. Iz Holter posmatranja, takođe, analizirani su ventrikularni poremećaji ritma. Kod bolesnika je obavljeno i kliničko praćenje, laboratorijski testovi i ehokardiografsko ispitivanje. **Rezultati.** Tokom perioda praćenja od godinu dana, 11 bolesnika je umrlo, od čega 10 zbog kardijalnog uzroka, a jedan usled cerebro-vaskularnog inzulta. Registrovane su značajno niže vrednosti SDNN (60,55 ± 12,84 ms *vs* 98,38 ± 28,21 ms), RRmax-RRmin (454,36 ± 111,00 ms *vs* 600,99 ± 168,72 ms) i prosečnog RR intervala (695,82 ± 65,87 ms *vs* 840,07 ± 93,97 ms) kod umrlih u odnosu na preživele ($p < 0.01$). Preminuli bolesnici bili su stariji, sa nižom ejekcionom frakcijom leve komore (0,46 ± 0,05 *vs* 0,56 ± 0,06), češćim kliničkim znacima srčane insuficijencije i ventrikularnim poremećajima ritma (> 10VES/h; $p < 0,01$). Multivarijantna Cox analiza pokazala je da je SDNN značajan, nezavisan prediktor za ukupni mortalitet kod bolesnika nakon infarkta miokarda. Drugi nezavisni prognostički faktori bili su klinički znaci srčane insuficijencije – Killip klasa II i III i učestale ventrikularne ekstrasistole. **Zaključak.** Smanjena varijabilnost srčane frekvencije je nezavisan prediktor mortaliteta kod bolesnika nakon infarkta miokarda i može pružiti korisne dodatne prognostičke informacije u neinvazivnoj stratifikaciji rizika kod ovih bolesnika.

Ključne reči:
infarkt miokarda; srce, frekvencija; aritmija; mortalitet; faktori rizika; testovi, prognostička vrednost.

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Introduction

The process of risk stratification following acute myocardial infarction (AMI) occurs in three stages: initial presentation, hospital course and assessment at hospital discharge [1]. It requires tests which can assess myocardial function, residual ischemia and propensity for ventricular arrhythmias. The most important determinant for mortality is left ventricular function, and left ventricular ejection fraction (LVEF) below 40% is a strong predictor of death in post-infarction patients [2]. Susceptibility to serious arrhythmias is reflected in ventricular ectopic activity, and other indicators of electrical instability such as depressed heart rate variability (HRV) or baroreflex sensitivity and an abnormal signal-averaged electrocardiogram [3, 4]. All of these can identify patients at increased risk of death. Depressed HRV in post-MI patients may reflect a decrease in vagal activity directed to the heart, which leads to prevalence of sympathetic mechanisms and to cardiac electrical instability.

The association of higher risk of post-infarction mortality with reduced HRV was first shown by Wolf et al. [5] in 1977. The variation of heart rate may be evaluated by a number of methods. The simplest to perform is the time domain measure of the standard deviation of all normal RR intervals (SDNN), which is most frequently used. More complicated are various spectral methods for the analysis of the tachogram – frequency domain methods. According to Task Force of the European Society of Cardiology and North American Society of Pacing and Electrophysiology, SDNN is recommended as a measure of HRV in post-infarction patients; SDNN is calculated over a 24-hour period from a continuous electrocardiographic (ECG) recording in patients before discharge from the hospital [6]. SDNN < 70 ms was used in several independent studies as a cutoff value defining normal and depressed HRV and a powerful predictor of mortality and arrhythmic complications in patients after AMI [2, 4]. The predictive value of HRV is independent of other factors established for post-infarction risk stratification, such as depressed LVEF, increased ventricular ectopic activity, and the presence of ventricular late potentials. In the era of modern treatment strategies that have modifying role in prognosis of post-MI patients, such as treatment with beta blockers, ACE inhibitors, revascularization and implantable cardioverter defibrillators (ICD), the prognostic significance of HRV has been challenged.

The aim of this study was to assess prognostic value of HRV in post-MI patients during the follow-up of one year.

Methods

The study was observational and prospective and included 110 patients with AMI who were admitted in the Coronary Care Unit of Clinical Center of Montenegro in Podgorica. Entry criteria were the diagnosis of first AMI with ST elevation, the age of 80 years or younger, and sinus rhythm. Exclusion criteria were atrial fibrillation or abnormal sinus node function, valvular disease or cardiomyopathy, and any other disease limiting survival. The study was approved by the Ethics Committee of the institution. Since the data obtained were non-invasive and did not exceed usual clinical management of the patients, the local Ethics Committee decided that signed informed consent was not needed. However, we did obtain oral informed consent.

Medical history, physical examination and laboratory findings were undertaken for all the patients. LVEF was measured within the two weeks of the index infarction by two-dimensional echocardiography. HRV and arrhythmia analysis (frequent ventricular premature complexes-VPCs > 10 VPCs/hour, VPC couplets and non-sustained ventricular tachycardia – NSVT) were measured by Holter-monitoring (24-hour ECG recording). Recordings were obtained from 8 to 13 days after an index infarction and contained at least 19 hours of analyzable ECG data including the whole night coverage. Biosensor Holter recorders were used. Tapes were analyzed for ventricular arrhythmias and HRV at the Holter laboratory of the Clinical Center of Montenegro by the published methods (a software package of Microsoft Corporation, after visual and manual editing of RR intervals and QRS complexes). The measure of HRV chosen for the primary analysis was SDNN calculated from 24-hour ECG recording, which represents measure of the global HRV. We also calculated simple time domain measures of HRV: RRmax-RRmin (difference between the longest RR interval – RRmax and the shortest RR interval – RRmin) and the mean RR interval (mean of all normal RR intervals). All the patients took their usual medications on the day of ECG recording.

The patients were followed-up for one year with visits every 6 months. All clinical events during the follow-up period were recorded and for patients who died we asked for the cause of death. The end-point of the study was all-cause mortality (death from any cause) and sudden cardiac death (SCD), which occurring instantaneous to 1 hour after the onset of a change in clinical status. In seven patients 24-hour ECG recording was not technically good for interpretation. We lost contact with three patients during the follow-up of one year. At the end of the study we had data of 100 patients for statistical analysis.

The arithmetic mean and standard deviation ($\bar{x} \pm SD$) were calculated for continuous variables. χ^2 and Independent Sample *t*-test were used to evaluate associations between the categorical and continuous variables. Statistical analysis was performed by using the statistical package SPSS 15.0 for Windows and data base in Microsoft Excel package. For identification of prognostic factors-predictors we used multivariate Cox analysis. A two-tailed *p* value of less than 0.05 was accepted as significant.

Results

We observed 110 patients, but at the end of the study we had data of 100 patients for statistical analysis. The mean age of patients was 56.99 ± 11.03 years, between them 80 were men and 20 women, mean BMI was 26.72 ± 3.44 kg/m^2. Anterior localization of AMI was diagnosed in 44% of the patients and inferior localization of AMI in 56% of the

patients. Physical examination found that 68% of the patients had Killip I class, 28% of them Killip II class and 4% of the patients had Killip III class during the stay in the Coronary Care Unit; mean LVEF was 0.54 ± 0.07. Analysis of HRV showed that mean SDNN was 94.22 ± 29.42 ms; 23% of the patients had SDNN < 70 ms; mean RR interval was 824.20 ± 101.71 ms and mean RRmax-RRmin was 584.86 ± 169.33 ms. Analysis of ventricular ectopic activity found that 16% of the patients had > 10 VPCs/hour. During a follow-up period of one year 11 patients died, 10 of them because of cardiac reason and one died of stroke. Among the patients who had cardiac death, 6 patients had SCD and 4 had worsening of congestive heart failure as a cause of death. Three patients died in the first 30 days after AMI, two patients had SCD and the third patient died because of congestive heart failure. Demographic and clinical characteristics of the patients are shown in Table 1. There was no difference in revascularization strategy between the patients who survived and the deceased patients, 51% of those treated with thrombolysis and no one with primary PCI ($p > 0.05$), but treatment with beta blockers was more frequently used in patients who survived ($p < 0.05$), without any differences in other medical therapy.

Analysis of HRV showed significantly lower values of SDNN (60.55 ± 12.84 ms *vs* 98.38 ± 28.21 ms), RRmax-RRmin (454.36 ± 111.00 ms *vs* 600.99 ± 168.72 ms) and the mean RR interval, (695.82 ± 65.87 ms *vs* 840.07 ± 93.97 ms) in the patients who died than in the survivors ($p < 0.01$) (Table 2).

The study findings showed depressed HRV – SDNN < 70 ms in 23 of the patients, and it was more frequent in the patients who died (9/11), than in those who survived (14/89; $p < 0.01$).

Ventricular ectopic activity, the number of ventricular premature complexes (VPCs) > 10/hour was more frequent in the patients who died than in the survivors ($p < 0.01$) (Table 3). There was no significant difference in the number of couplets and episodes of NSVT during Holter monitoring in patients who died compared to patients who survived ($p > 0.05$).

Table 1

Demographic and clinical characteristics of the study population

Characteristics of patients	Survived (n = 89)	Dead (n = 11)	*p*
Age (years), $\bar{x}$ ± SD	58.78 ± 10.82	62.83 ± 7.35	< 0.01
Male/Female (n)	72/17	8/3	> 0.05
BMI (kg/m^2), $\bar{x}$ ± SD	26.62 ± 3.34	27.24 ± 4.21	> 0.05
Smoker (n)	63	7	> 0.05
Hypertension (n)	44	6	> 0.05
Hyperlipidemia (n)	27	3	> 0.05
Diabetes mellitus (n)	14	2	> 0.05
Heart rate at admission (b.p.m.), $\bar{x}$ ± SD	82.78 ± 21.26	79.82 ± 28.81	> 0.05
Systolic blood pressure (mmHg), $\bar{x}$ ± SD	138 ± 22	142 ± 24	> 0.05
Diastolic blood pressure (mmHg), $\bar{x}$ ± SD	89 ± 14	93 ± 12	> 0.05
Hemoglobin (g/l), $\bar{x}$ ± SD	141 ± 10	136 ± 12	> 0.05
Glucose (mmol/L), $\bar{x}$ ± SD	7.42 ± 1.25	7.15 ± 1.43	> 0.05
Creatinine (μmol/L), $\bar{x}$ ± SD	82.38 ± 29.24	85.14 ± 31.18	> 0.05
Total cholesterol (mmol/L), $\bar{x}$ ± SD	6.21 ± 1.33	6.06 ± 1.42	> 0.05
LDL- cholesterol (mmol/L), $\bar{x}$ ± SD	4.22 ± 1.24	3.98 ± 1.45	> 0.05
HDL- cholesterol (mmol/L), $\bar{x}$ ± SD	1.14 ± 0.21	1.20 ± 0.31	> 0.05
Triglycerides (mmol/L), $\bar{x}$ ± SD	2.01 ± 1.12	1.93 ± 1.23	> 0.05
Sodium (mmol/L), $\bar{x}$ ± SD	138.11 ± 2.24	139.39 ± 2.95	> 0.05
Potassium (mmol/L), $\bar{x}$ ± SD	4.32 ± 0.43	4.16 ± 0.36	> 0.05
Creatin kinase Mb peak (IU/L), $\bar{x}$ ± SD	100.44 ± 83.40	118.39 ± 72.37	> 0.05
Killip class I / II / III (n)	67 / 20 / 2	1 /8 / 2	< 0.01
Left ventricular ejection fraction (%), $\bar{x}$ ± SD	0.56 ± 0.06	0.46 ± 0.05	< 0.01

BMI – body mass index; LDL – low density lipoprotein; HDL – high density lipoprotein.

Table 2

Heart rate variability and mortality

Parameter	Survived $\bar{x}$ ± SD	Dead $\bar{x}$ ± SD	*p*
SDNN (ms)	98.38 ± 28.21	60.55 ± 12.84	< 0.001
Mean RR interval (ms)	840.07 ± 93.97	695.82 ± 65.87	< 0.001
RRmax-RRmin (ms)	600.99 ± 168.72	454.36 ± 111.00	0.006

SDDN – standard deviation of all normal RR intervals.

Table 3

Ventricular ectopic activity (> 10 VPCs/hour) and mortality

> 10 VPCs / h	Survived	Dead	χ^2	*p*
Yes	11	5	7.978	0.009
No	78	6		

VPC – ventricular premature complex.

Multivariate Cox analysis showed that SDNN was a significant, independent predictor of all-cause mortality and cardiac mortality in post-infarction patients ($p < 0.01$), but SDNN was not an independent predictor of SCD ($p > 0.05$). Also the independent predictors of all-cause mortality and cardiac mortality were the clinical signs of heart failure – Killip class II and III, ($p < 0.01$) and a ventricular ectopic activity (number of VPCs > 10/hour; $p < 0.05$) (Table 4). The independent predictors of SCD were the mean heart rate (RR 1.14; CI 1.04–1.26, $p = 0.0053$) and the ventricular ectopic activity (the number of VPCs > 10/hour) at Holter monitoring (RR 17.84; CI 2.03–156.51, $p = 0.0093$); the higher mean heart rate was associated with the higher risk for SCD.

Table 4

Cox analysis of the predictors of all-cause mortality

Variable	B	S.E.	Wald	df	*p*	*r*	EXP (B) RR	95% CI for EXP(B)
Killip class	2.31	0.83	7.79	1	0.0052	0.25	10.11	1.99–51.27
SDNN	-0.12	0.03	13.57	1	0.0002	-0.36	0.89	0.84–0.95
> 10 VPCs/h	1.78	0.72	6.10	1	0.0135	0.21	5.90	1.44–24.16

SDNN – standard deviation of all normal RR interval; VPC – ventricular premature complex.

Discussion

Prediction of fatal events in patients following AMI is very difficult, especially non-invasive risk stratification. The assessment of left ventricular function, use of signal-averaged electrocardiography and Holter monitoring were shown to be predictive for future mortality and arrhythmic events in patients after AMI. There is a clear progressive increase in mortality, especially as the LVEF falls < 40% [2]. Multivariate analysis showed that VPCs frequency and complexity are also an independent risk factor for total cardiac mortality and SCD [7–9].

Our study provides evidence that, beside clinical signs of heart failure and frequent VPCs, HRV has independent prognostic value in post-MI patients. We demonstrated that SDNN value was independently inversely associated with the risk of all-cause mortality after an acute myocardial infarction with ST segment elevation, but not with the risk of SCD. We showed that increased mean heart rate and frequent ventricular ectopy on post-infarction Holter recording independently predicted increased risk of SCD. The knowledge of a patient's autonomic status improves risk stratification over and beyond that obtained from the established clinical predictors such as clinical signs of heart failure, LVEF, and ventricular arrhythmias. It has become clear that autonomic nervous system is very important in the pathogenesis of ventricular arrhythmia and death.

Kleiger et al. [10] pointed that HRV had the strongest univariate correlation with mortality in 808 post-infarction patients. Patients with reduced HRV had higher mortality, independent of the LVEF. Patients with a SDNN < 50 ms had a relative risk of all-cause mortality of 2.8. These studies showed that HRV was an independent predictor of death additive to other post-infarction risk variables, such as LVEF and heart rate. Our study showed that the mean heart rate at Holter monitoring was an independent predictor of SCD additive to frequent ventricular ectopic activity (> 10 VPCs/hour).

The results of GISSI-2 study demonstrated that SDNN < 70 ms was an independent predictor of cardiac mortality in patients after myocardial infarction [11]. The findings of ATRAMI study showed that low baroreflex sensitivity and low SDNN contributed to high risk of cardiac mortality in post-MI patients and that an altered cardiac substrate, identified by depressed LVEF and by the presence of frequent VPCs, was not the sole significant predictor of post-infarction cardiac mortality [4]. These findings pointed to the critical role of an altered autonomic balance that resulted in a relatively high sympathetic activity and a low vagal activity as showed by low values of baroreflex sensitivity and SDNN.

A meta-analysis of 51 trials included 3,489 post-infarction patients with an overall mortality of 125/577 (21.7%) in patients with SDNN < 70 ms compared to 235/2912 (8.1%) in patients with SDNN > 70 ms. The meta-analysis demonstrated that, after a myocardial infarction, patients with SDNN < 70 ms had almost four times more chance to die in the next 3 years than those with SDNN > 70 ms [12, 13]. The results of a CARISMA study suggest that fatal or near-fatal arrhythmias can be predicted by many risk stratification methods, especially by heart rate variability, in patients with reduced LVEF after AMI [14].

Our findings showed significantly lower SDNN in patients who died than in those who survived, mortality was higher in patients with SDNN < 70 ms, and depressed HRV was an independent predictor of all-cause mortality and cardiac mortality in post-MI patients in addition to the clinical signs of heart failure and frequent VPCs. Treatment with beta blockers was more frequently used in patients who survived, without a difference in revascularization strategy and other medical therapy. This observation might explain higher SDNN and better prognosis in patients treated with beta blockers. These drugs possibly can increase HRV. The decrease in sympathetic activity was noticed in post-infarction patients using metoprolol [15] and in patients with heart failure using acebutolol [16]. Thus, beta blockers are able to restore the sympathetic–parasympathetic balance in cardiovascular disease. The deceased patients in our study were of higher mean age and with lower left ventricular ejection fraction, too.

Risk stratification of post-infarction patients is important since the preventive therapy with ICD is effective in re-

ducing mortality. The guidelines for primary prevention with ICD in post-infarction patients basically use LVEF for risk stratification (at least 40 days after AMI) [17, 18]. However, many deaths occur in low-risk patients with normal LVEF who do not fulfill criteria for ICD implantation, and some patients with an ICD do not appear to gain benefit from the device [19–21]. There is no ideal particular test for risk stratification in patients following AMI. There is a need for combination of tests [22–26]. The findings of some studies showed combination of LVEF ≤ 40% + reduced HRV + frequent VPCs on Holter monitoring had positive predictive value of 50% [9]. For example in a DINAMIT study, designed to assess the impact of ICD implantation on top of optimal medical therapy (OMT) *versus* OMT alone on all-cause mortality in high-risk patients within 40 days after AMI, the inclusion criteria were the occurrence of AMI 6–40 days prior to enrollment, LVEF ≤ 35%, SDNN ≤ 70 ms and the mean RR interval ≤ 750 ms (the last three criteria were used for stratification of high-risk patients) [27]. A review of the literature suggested staged combination of tests, with Intracardiac Electrophysiology Study (EPS) last, which allowed 91.8% of patients to be stratified as either high or low risk [24, 28]. Further investigations will be needed to determine the most useful predictive combination of tests for risk stratification in post-infarction patients.

The limitations of our study include a small number of patients and a small number of events in each group and therefore study could be underpowered to detect long-term mortality difference.

Conclusion

This study shows that depressed heart rate variability is an independent predictor of mortality in post-infarction patients additive to clinical signs of heart failure and ventricular ectopic activity and may provide useful additional prognostic information in non-invasive risk stratification. These findings suggest that in post myocardial infarction patients reduction in vagal activity, which is almost always accompanied by a concomitant increase in sympathetic activity, is associated with higher risk for death. Heart rate variability is simple, non invasive and relatively not expensive to obtain.

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Conflicts of interest

The authors declare no conflict of interest.

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The influence of bacterial vaginosis on gestational week of the completion of delivery and biochemical markers of inflammation in the serum

Uticaj bakterijske vaginoze na nedelju završetka porođaja i biohemijske markere inflamacije u serumu

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Abstract

Bacground/Aim. Preterm delivery is one of the most common complications in pregnancy, and it is the major cause (75–80%) of all neonatal deaths. Bacterial vaginosis predisposes to an increased risk of preterm delivery, premature rupture of membrane and miscarriage. In this syndrome normal vaginal lactobacilli, which produce protective H_2O_2, are reduced and replaced with anaerobic, gram-negative bacteria and others. The aim of this study was to evaluate the influence of bacterial vaginosis on the week of delivery and biochemical markers of inflammation in the serum. **Methods.** A total of 186 pregnant women were included into this study, between the week 16 and 19 of pregnancy. In the study group there were 76 pregnant women with diagnosed bacterial vaginosis by the criteria based on vaginal Gram-stain Nugent score and Amsel criteria. In the control group there were 110 healthy women with normal vaginal flora. Ultrasound examination was performed in both groups. Vaginal fluid and blood samples were taken to determine biochemical markers with colorimetric methods. **Results.** The week of delivery was statistically significantly shorter in the study group and the levels of biochemical markers of inflammation (C-reactive protein and fibrinogen in the serum) were statistically significantly higher in women with bacterial vaginosis comparing to the control group. Also the levels of uric acid and white blood cells in the serum were higher in the study group compared to the control one. **Conclusion.** Our study indicates that the pregnancy complicated with bacterial vaginosis ends much earlier than the pregnancy without it. Also, higher levels of biochemical markers of inflammation in the serum in the study group, similarly to results of other studies, suggest that pathophysiological processes responsible for preterm delivery can begin very early in pregnancy.

Key words:
pregnancy; vaginosis, bacterial; premature birth; risk factors; biological markers.

Apstrakt

Uvod/Cilj. Prevremeni porođaj predstavlja jednu od najčešćih akušerskih komplikacija, i kod 75–80% slučajeva uzročnik je neonatalnog mortaliteta. Bakterijska vaginoza smatra se značajnim faktorom rizika od prevremenog porođaja, prevremene rupture plodovih ovojaka i pobačaja. Nastaje kao posledica redukcije laktobacila koji proizvode protektivni H_2O_2, sa preovladavanjem anaerobnih, gram-negativnih i drugih nepovoljnih bakterija. Cilj ovog rada bio je da se utvrdi da li bakterijska vaginoza kod trudnica ima uticaja na nedelju završetka porođaja kao i na biohemijske markere inflamacije u serumu. **Metode.** Studija je obuhvatila ukupno 186 trudnica, između 16. i 19. nedelje gestacije. Ispitanice su bile podeljene na grupu sa dokazanom bakterijskom vaginozom po Amselovim i Njudžentovim kriterijumima (n = 76) i kontrolnu grupu sa normalnom bakterijskom florom (n = 110). Svim trudnicama je urađen ultrazvučni pregled, uzet bris vaginalnog sekreta i uzorak krvi za određivanje biohemijskih markera inflamacije kolorimetrijskim metodama. **Rezultati.** Nedelja završetka porođaja bila je statistički značajno kraća kod trudnica sa bakterijskom vaginozom u odnosu na kontrolnu grupu, dok su biohemijski markeri inflamacije, C-reaktivni protein i fibrinogen, bili statistički značajno viši u ispitivanoj grupi nego u kontrolnoj grupi. Takođe, vrednosti mokraćne kiseline i ukupnih leukocita bile su više u ispitivanoj u odnosu na kontrolnu grupu. **Zaključak.** Naša istraživanja ukazuju na znatno raniji završetak porođaja kod trudnica sa bakterijskom vaginozom, kao i na to da više vrednosti biohemijskih markera inflamacije u serumu ispitivane grupe, slično drugim istraživanjima, sugerišu da se patofiziološki procesi odgovorni za prevremeni porođaj mogu javiti vrlo rano u trudnoći.

Ključne reči:
trudnoća; vaginoza, bakterijska; porođaj, prevremeni; faktori rizika; biološki pokazatelji.

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Introduction

Bacterial vaginosis (BV) presents the adverse of vaginal ecosystem, which results in decreasing or complete disappearance of hydrogen-peroxyde producing lactobacillus and enormous increase of anaerobic facultative bacteria which are 100 to 1,000 more than usual [1–4]. In non-pregnant women BV increases risk of pelvic inflammatory disease (PID), postabortional PID, postoperative infections and pathological cervical changes, while in pregnancy there is higher frequency of premature rupture of membranes (PROM), preterm delivery, chorioamnionitis or postpartal endometritis. It is assumed that BV is present in 30% of pregnant women [4, 5]. In the last 20 year, extensive studies indicated close relationship between BV and preterm delivery, which presents one of the most important causes of perinatal morbidity and mortality [6–9]. The exact mechanism of BV leading to preterm delivery is still unknown, considering that its main feature is the absence of signs of inflammation, low production of cytokines and the absence of inflammatory cells such as macrophags and neutrophils [8–10]. However, meta-analyses indicate that as soon as BV is diagnosed [< 20 gestational weeks (GWs)] the risk of preterm delivery is increased [11–13]. It is assumed that the risk of pretern delivery is associated with the type of vaginal flora and also with the type of immune answer that controls inflammatory process whose background could be found in genetic explanations. The attitude of some authors [5, 10, 14] is that asymptomatic pregnant women with previous premature deliveries or abortions in second trimester of pregnancy, should control, and, in case of occurence of subjective complaints, cure with vaginalets, and, in case of positive bacterial smears, systemic antibiotic therapy should be applied.

The aim of this study was to determine a connection between BV and the week of delivery completion, and also to investigate if early detected BV (< 20 GW) can affect biochemical markers of inflammation in the serum, in order to evaluate the pathophysiological pathways by which BV leads to preterm delivery.

Methods

This prospective study was conducted in the Department of Gynecology and Obstetrics, Clinical Center of Vojvodina, Novi Sad. The study included 198 pregnant women in total who agreed to participate in research, and they confirmed it with their signature in accordance with the Helsinki Declaration. The protocol was approved by the Ethics Committee of the Medical Faculty in Novi Sad and Clinical Center of Vojvodina, Novi Sad, Serbia.

All the pregnant women included in the research (gestational age between the GW 16 and 19 had an ultrasound examination to assess gestational age, fetal growth and development and viability of fetus. After that swabs of vaginal secretion and blood samples were taken. All the pregnant women were included in the study and monitored until the end of pregnancy.

All the participating women were divided into two groups: the study group (n = 80) consisted of pregnant women with BV diagnosed by the Amsel (score ≥ 3) and Nugent criteria (score ≥ 7) [15, 16], and the control group of pregnant women with normal vaginal flora. Pregnant women with the diagnosed intermediate vaginal flora were excluded from the research. During monitoring 12 women in total were excluded from the research. One pregnant woman had a spontaneous abortion in GW 17, one woman in GW 22 was diagnosed with fetal anomalies, and one pregnant woman had preterm delivery due to oligohydramnion. Total of 9 pregnant women were excluded due to inability of monitoring (or did not appear in Clinical Center of Vojvodina or we could not get information by telephone). Finally, the control group included of 110 pregnant women, and the study group included 76 pregnant women. Other factors that could lead to preterm delivery were the criteria for exclusion of pregnant women from the research. These factors were: multiple pregnancy, polyhydramnion, placenta praevia, diseases of mother and fetus (diabetes, hypertension, preeclampsia, eclampsia, kidney and heart diseases of mother, urinary infections, genetic malformations of fetus, intrauterine growth retardation), the local factors: anatomical malformations of uterus and vagina, cervical insufficiency, other genital infections, uterine tumors, then all diseases that can affect the level of biochemical markers in the serum such as autoimmune diseases and hormone disbalance, and also pregnant women younger than 18 and use of antibiotics just before conception and during pregnancy.

Vaginal swab was taken from lateral wall of vagina and used to create direct preparation stained by Gram and scored by Nugent method and Amsel method for diagnosis of BV [14–16].

Hematological parameters were determined on an automatic hematological analyzer ABX Micros CRP200 (HoribaABX Diagnostics). On the same machine concentration of C-reactive protein (CRP) was determined by nephelometric method.

Glucose in the serum was determined by the enzyme referent method with hexokinase with commercial reagent (Roshe Diagnostics) on a biochemical analyzer Cobas Integra 400 plus. Uric acid was determined by the enzyme colorimetric method with uricase and peroxidase with commercial reagent (Roshe Diagnostics) on a biochemical analyzer Cobas Integra 400 plus.

Fibrinogen concentration was determined on a BFT II Fibrintimer (Siemens Health Care Diagnostics) with a modified method by Klaus with Multifibren U-reagent.

Statistical analysis was done by statistical package SPSS (ver.13) for Windows, and *p* value less than 0.05 were considered statistically significant. Student's *t*-test and Mann-Whitney test were used to compare variables between the two groups.

Results

The study included 76 pregnant women of the study group, aged between 20 and 41, and 110 pregnant women of the control group, aged between 19 and 42. Table 1 shows characteristics of all the pregnant women included in the re-

Table 1

Age of pregnant woman, gestational week (GW) during swab sample taking and the week of pregnancy completion (CW)

Parameter	Study group (n = 76)	Control group (n = 110)	*p*
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
Age (years)	31.47 ± 5.51	31.44 ± 5.86	0.974
GW	17.50 ± 0.808	17.41 ± 0.670	0.489
CW	37.72 ± 3.948	39.59 ± 1.06	< 0.001*

***Statistically significant defference.**

search. There was a statistically significant difference between the groups in the week of completion of pregnancy ($p < 0.001$), while there was no statistically significant difference between the age and the weeks of gestation during swab sampling.

Table 2 shows percentage ratio of vaginal deliveries (VG) and Cesarean sections (CS) in the study and the control group.

Table 2

Mode of delivery

Group	Mode of delivery		Total
	VG	CS	
Control, n (%)	85 (77.1)	25 (22.9)	110 (100)
Study, n (%)	57 (75.0)	19 (25.0)	76 (100)
Total, n (%)	142 (100)	44 (100)	186 (100)

VG – vaginal delivery; CS – Cesarean sections.

Table 3 shows comparison of the mean values of uric acid, glucose, CRP, fibrinogen and hematological parameters in blood between the study and the control group. The values of CRP and fibrinogen were statistically significantly higher in pregnant women with BV compared to the control group ($p < 0.001$).

mature birth?“, “Why is BV detected in early pregnancy more associated with preterm delivery than the one diagnosed later in pregnancy?” [21–24]. It is assumed that one of the main factors that makes finding the answers to these questions difficult, lies in the name of the syndrome “vaginosis” and not vaginitis which explains that there is the absence of vaginal inflammatory process [2, 19]. It is assumed that there are several factors that can individually or jointly increase the risk of preterm delivery. Microorganisms that cause BV can ascendantly spread from lower parts of genital tract to upper parts and lead to chorioamnionitis, a preterm rupture of fetal membranes and preterm delivery [3, 25, 26]. Then, microorganisms can produce proteolytic enzymes that increase epithelium permeability in vagina, and allow passage of very pathogenic microorganisms [27, 28]. Results of other researches indicate the role of local immunological factors, genetically predisposed and depending on their presentation to microorganisms causing BV, local inflammatory mediators such as cytokines, chemokines, and growth factors that determine what kind of consequences will appear. It is also assumed that the increased production of local cytokines-prostaglandins, can trigger preterm delivery [29–32].

Table 3

Mean values of hematological parameters, C-reactive protein (CRP), fibrinogen, uric acid and glucose

Variables	Study group (n = 76)	Control group (n = 110)	*p*
	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
Uric acid (μmol/L)	170.43 ± 110.81	150.33 ± 96.84	0.520
Glucose (mmol/L)	2.99 ± 1.71	2.83 ± 1.82	0.768
CRP (mg/L)	7.07 ± 6.93	2.60 ± 1.50	< 0.001*
Fibrinogen (g/L)	4.44 ± 0.52	4.12 ± 0.36	< 0.001*
Leucocytes (x 10^9/L)	8.75 ± 1.69	8.14 ± 1.75	0.129
Erythrocytes (x 10^{12}/L)	3.96 ± 0.42	4.04 ± 0.41	0.408
Thrombocytes (x 10^9/L)	225.57 ± 52.73	204.91 ± 53.61	0.098
Hemoglobin (g/L)	114.80 ± 9.19	118.63 ± 13.78	0.151

***statistically significant difference.**

Discussion

BV is a syndrome resulting in a significant reduction of lactobacilli necessary for the creation of H_2O_2, which enables propagation of *Gardanella vaginalis*, *Mycoplasma hominis*, anaerobes etc. [17–20]. There is no doubt that BV and preterm delivery are associated. Currently, a number of studies have been aimed at finding answers to questions: “What are pathophysiological mechanisms linking BV and preterm delivery?”, and “Why are a large number of pregnancies, regardless of whether BV is diagnosed by clinical or microscopic methods, spontaneously resolved without consequences for mother or child and only 10–15% have a pre-

Metronidazole is the drug of choice for bacterial vaginosis. However, application of imidazole derivatives in pregnancy is still debatable, although recent studies indicate that there is no evidence of teratogenicity of this drug; it is still necessary to estimate the maternal benefit in relation to fetal-neonatal risk [6]. It has been shown that treatment with metronidazole in a dose of 400 mg 2 times a day for 5 days, and of 500 mg 2 times a day for 7 days, is more effective than giving 2 g in a single dose. Intravaginal metronidazole gel (0.75%) and clindamycin intravaginal cream (2%) have similar efficiency, because theoretically metronidazol is less active to lactobacilli than clindamycin, while clindamycin is more active to the most of the bacteria

that are related to BV [33]. However, peroral application of clindamycin can be accompanied with appearance of maternal pseudomembranous colitis, which is more often in-pregnant than in non-pregnant women. Application of 2% klindamycin vaginal cream one time a day is suggested, while in second and third semester of pregnancy such women can apply antibiotic therapy with: 500 mg metronidazol 2 times for 7 days, or 400 mg metronidazol 2 times for 2 days (if necessary, repeat after 4 weeks) and 250 mg of metronidazol i 333 mg erythromycina 3 times for 7 days. The treatment reduces the incidence of preterm delivery in pregnant women with increased risk (history of preterm deliveries in previous pregnancies) [33]. A group of authors [33] from Medical Faculty in Wroclaw, Poland, in their reserach from 2006 determined the importance of using hydrophilic vaginal tablets containing complex of lactic acid and Eudragit E-100. It turned out that these vaginal tablets are very useful in therapy of symptoms of BV in pregnant women. During the therapy there were no side effects, and after the therapy vaginal mucosa did not show signs of irritation or allergic reactions [34–36].

The results of our study agree are in accordance with those in the literature [1, 7, 16, 28]. There is a statistically significant difference in week of pregnancy completion in women with BV compared to women with normal vaginal flora, while the serum levels of biochemical markers of inflammation, CRP and fibrinogen, also show statistically significantly higher values in the study group than in the control one. The values of uric acid and total number of leukocytes are higher in the study than in the control group. There is also a higher number of Cesarean deliveries in the group with BV than in the control group.

Conclusion

The results of our study indicate a significantly earlier delivery in pregnant women with the diagnosed bacterial vaginosis in early pregnancy, and also statistically significantly higher values of C-reactive protein and fibrinogen in pregnant women with bacterial vaginosis, that can help in further research with both existing and possible new markers, which could help in clarification of pathogenetic mechanisms which link bacterial vaginosis and preterm delivery, as well as in identification of women with the risk of preterm delivery caused by infection, at the same time. However, it is also necessary to expand existing researches and include genetic analysis, i.e. examine the response of genotype and immunologically different phenotypes in dependence on change of microenvironment or inflammation, in order to explain pathophysiology of preterm delivery.

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Prediction of mortality with unmeasured anions in critically ill patients on mechanical ventilation

Predviđanje mortaliteta neizmerenim anjonima kod kritično obolelih na mehaničkoj ventilaciji

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Abstract

Background/Aim. Acid-base disorders are common within critically ill patients. Physicochemical approach described by Stewart and modified by Figge gives precise quantification method of metabolic acidosis and insight into its main mechanisms, as well as influence of unmeasured anion on metabolic acidosis. The aims of this study were to determine whether the conventional acid-base variables are connected with survival rate of critically ill patients at Intensive care unit; whether strong ion difference/strong ion gap (SID/SIG) is a better predictor of mortality rate comparing to conventional acid-base variables; to determine all significant predictable parameters for the 28-day mortality rate at intensive care units. **Methods.** This retrospective observational analytic study included 142 adult patients requiring mechanical ventilation, survivors (n = 68) and nonsurvivors (n = 74). Apparent strong ion difference (SIDapp), effective strong ion difference (SIDeff) and SIG values were calculated with the Stewart-Figge's quantitative biophysical method. Descriptive and analytical statistical methods were used in the study [*t*-test, Mann-Whitney U test, χ^2-test, binary logistic regression, Reciever operating characteristic (ROC) curves, calibration]. **Results.** Age, Na^+, acute physiology and chronic health evaluation (APACHE II), Cl^-, albumin, SIG, SID app, SIDeff, and aninon gap (AG) were statistically significant predictors. AG represented a model with imprecise calibration, i.e. a model with little predictive power. APACHE II had *p*-value more than 0.05 if it was near it, and therefore it could be considered potentially unreliable for outcome prediction. SIDeff and SIG represented models with well-defined calibration. ROC analysis results showed that APACHE II, Cl^-, albumin, SIDeff, SIG i AG had the largest area bellow the curve. By creation of logistic models with calibration methods, we found that outcome depends on SIG and APACHE II score. **Conclusion.** Based on our data, unmeasured anions provide prediction of mortality of critically ill patients on mechanical ventilation, unlike the traditional acid-base variables which are not accurate predictors of the 28-day mortality rate.

Key words:
critical illness; acid-base imbalance; intensive care units; mortality.

Apstrakt

Uvod/Cilj: Acidobazni poremećaji su uobičajeni kod kritično obolelih. Fizičko-hemijski pristup koji je opisao Stewart a modifikovao Figge omogućava precizan način kvantifikovanja metaboličke acidoze i pruža uvid u njene glavne mehanizme, kao i doprinos neizmerenih anjona metaboličkoj acidozi. Ova studija imala je za cilj da utvrdi: da li su konvencionalne acidobazne varijable povezane sa mortalitetom kritično obolelih u jedinici intenzivne nege; da li su snažna jonska razlika/snažni jonski gap (SID/SIG) bolji prediktori mortaliteta od konvencionalnih acidobaznih varijabli; sve značajne prediktivne faktore acidobazne ravnoteže za 28-dnevni mortalitet u jedinicama intenzivne nege. **Metode.** Ovom retrospektivnom opservacionom analitičkom studijom bila su obuhvaćena 142 odrasla bolesnika na mehaničkoj ventilaciji od kojih je preživelo 68 i umrlo 74. Vrednosti očigledne snažne jonske razlike (SIDapp), efektivna snažna jonska razlika (SIDeff) i SIG izračunavane su pomoću Stewart's-Figge kvantitativnog biofizičkog metoda. Korišćene su deskriptivne i analitičke statističke metode [*t*-test, Mann-Whitney *U*-test, χ^2-test, binarna logistička regresija, (*Receiver operating characteristic* – ROC) krive, kalibracija]. **Rezultati.** Univarijantna analiza ukazuje da su starost, Na^+, APACHE II, Cl^-, albumin, SIG, SIDapp, SIDeff i anjonski gap (AG) statistički značajni prediktori. AG se pokazao kao model sa lošom kalibracijom, odnosno model sa malom prediktivnom moći. APACHE II imao je *p* vrednost neznatno veću od 0,05, pa se i on može smatrati potencijalno sumnjivim za predikciju ishoda. SIDeff i SIG su se pokazali kao modele sa dobrom kalibracijom. ROC analiza je ukazala da APACHE II, Cl^-, albumin, SIDeff, SIG i AG imaju najveću površinu ispod krive. Kreiranjem logističkih modela metodom kalibracije pronašli smo da ishod zavisi od SIG i APACHE II skora. **Zaključak.** Dobijeni podaci pokazuju da neizmereni anjoni omogućavaju predviđanje mortaliteta kritično obolelih na mehaničkoj ventilaciji, za razliku od tradicionalnih acidobaznih varijabli koje nisu precizni prediktori 28-dnevnog preživljavanja.

Ključne reči:
kritična stanja; acidobazna ravnoteža, poremećaji; intenzivna nega, odeljenja; mortalitet.

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Introduction

Acid-base disorders are common in critically ill patients [1]. Traditional measurements which allow partial quantification of metabolic component of acid-base disorders are the following: pH, anion gap (AG), standard bicarbonates (SB), and standard base excess (SBE) [2]. Anion gap is the term used for apparent lack of anions compared to cations. This anion shortage in healthy persons is only apparent because only electrolytes of vital importance (sodium, potassium, chlorides and bicarbonates) are measured. If wider an anion gap, it indicates the presence of additional anions in plasma, such as: ketones, lactates, acid interproduct in salicylic acids, methanol or paraldehyde poisoning. In other words, anion gap widening is an indication for acidosis [3].

Numerous studies show, however, that conventional parameters of metabolic status have limited accuracy in predicting the outcome of treatment and the percent of mortality of critically ill patients [4–7]. The reasons for limited precision probably originate in different mechanisms involved in acid-base disorders formation: cumulative effect of hypoalbuminemia (values less than 35g/L), influence of various metabolites of unmeasured anions, the presence of various types of acidosis, the degree of hyperlactatemia (lactates values more than 2 mmol/L) [4, 7, 8]. The physicochemical approach described by Stewart [9] and modified by Figge et al. [10] gives a precise quantification method of metabolic acidosis. Also, it gives insight into its main mechanisms, as well as the influence of unmeasured anion on metabolic acidosis. This approach emphasizes that changes in blood pH are regulated by three independent variables: pH, strong ionic difference (SID) and total weak acids concentration [9, 10].

The partial pressure of carbon-dioxide ($PaCO_2$) provides some information about the respiratory component of acid-base disorders. However, the interpretation of metabolic component is far more complex. Apparent strong ion difference (SIDapp) is a difference between the sum of all strong cations and strong anions measured [9, 10]. Effective strong ion difference (SIDeff) represents the effect of corrected $PaCO_2$, weak acids (albumins), and inorganic phosphates on electric charge balance in plasma [10]. The difference between SIDapp and SIDeff measured represents a strong ion gap (SIG) [11]. The SIG value for healthy people is zero, while within critically ill patients high SIG is defined by the values ≤ 2 and indicates accumulation of unmeasured anions (sulfate, keto acids, citrate, pyruvate, acetate, gluconate, etc.) [8, 10–14]. Unmeasured anions are a sign of acidosis that must be included to account for the measured pH [14–19].

The aim of this study was to determine whether the conventional acid-base variables are connected with survival rate of critically ill patients at intensive care units (ICU), whether SID/SIG is a better predictor of mortality rate comparing to conventional acid-base variables, as well as to determine all significant predictable parameters for the 28-day mortality rate at Intensive care unit.

Methods

This retrospective observational analytic study involved subpopulation of critically ill patients on mechanical ventilation, admitted to the Intensive Care Unit, during the period January 2012–October 2012. The study was approved by the institutional ethical commitee.

Inclusion criteria in the study were the following: patients who needed mechanical ventilation and intensive monitoring of vital parameters (ECG monitoring, body temperature, arterial blood pressure). It was necessary that arterial gas analyses and biochemical analyses were done on admission date at Intensive Care Unit (electrolytes, albumins, haematocrit, leukocytes, and creatinine). Exclusion criteria were: patients under 18, patients admitted due to various poisoning, and patients diagnosed with cancer. According to the outcome, patients were divided into two groups: survivors and nonsurvivors. Fluid resuscitation was performed with crystalloids, colloids and blood products, according to the diagnosis of critically ill. All the patients were monitored during a 28-day period from the moment of admission to Intensive Care Unit in order to establish mortality rate [13].

Demographic data, admission diagnosis, APACHE II score values within the first 48 hours of admission (Acute Physiology And Chronic Health Evaluation), and treatment outcome (survivors and nonsurvivors) were collected from case histories and discharge notes of patients involved in the study. Venous blood was collected through a cannula introduced for therapy application. Different veins in forearm were drawn. Arterial blood was sampled from radial artery. Arterial puncture was done with syringe and needle (24–26 G) which were covered with heparin as anticoagulant. All samples were analysed with a gas analyser (GEM Premier 3000, Instrumentation Laboratory, Italy). Biochemical parameters were analysed by biochemical analyser (Ilab 600, Instrumentation Laboratory, Italy).

AG values were calculated with the following formula: $AG = [Na^+ + K^+] - [Cl^- + HCO_3^-]$, (concentrations are in mmol/L) [2, 3].

SIDapp, SIDeff and SIG values were calculated with the Stewart-Figge's quantitativebiophysical method using the following formulas:

$SIDapp = [Na^+ + K^+ + Ca^{2+}] - [Cl^- + \text{lactate}]$, (my concentrations are in mmol/L) [9, 10].

$SIDeff = 2.46 \times 10^{-8} \times PaCO_2/10^{-pH} + [\text{albumin}] \times (0.123 \times pH - 0.631) + (0.309 \times pH - 0.469)$ [10]. In this equation, PaCO2 is measured in kPa, albumin in g/L.

$SIG = SIDapp - SIDeff$ [11].

The minimum data required by a calculator of unmeasured anions is: pH, $PaCO_2$, Na^+, K^+, Cl^- and albumins [13].

The following descriptive methods were used: absolute and relative numbers, central trend measures (arithmetic mean and median), and dispersion measures (SD – standard deviation). Comparison tests (*t*-test, Mann-Whitney *U*-test, χ^2-test), and correlation analysis (binary logistic regression) were used as analytical methods. Receiver operating characteristic (ROC) curves were created in order to estimate which

variables analysed have mortality discriminating prediction, as well. Any analyses with $p < 0.05$ were considered relevant. The accuracy of treatment outcome prediction with prognostic model was shown by the calibration Hosmar-Lemeshow test (H-L test). This test assesses whether or not the observed event rates match the expected event rates in the subgroups of the model population [15]. SPSS 12.0 software package (Chicago, Illinois) was used for statistical analysis.

Results

There were 142 subjects included in the study, 67 men and 75 women. The patients were divided into two categories according to the 28-day survival rate: survivors (n = 68) and nonsurvivors (n = 74). The average age in the survivors group was 56.43 ± 17.45 years and nonsurvivors 64.05 ± 15.77 years. Detailed information about the average values and the results of logistic regression for survivors and nonsurvivors groups are given in Table 1. Univariate analysis showed that the following predictors are statistically significant: age, Na^+, APACHE II, Cl^-, albumin, SIG, SID app, SIDeff and AG. Nevertheless, for all the models it is important to emphazise that AG is a model with poor calibration, or a little predictive power. APACHE II had *p*-value more than 0.05 if it was near it, and therefore it could be considered potentially unreliable for outcome prediction. On the other hand, SIG was a model with well-defined calibration. The results of ROC analysis (Table 2) shoed that statistically significant predictors were as follows: age, Na^+, APACHE II,

Table 1

Demographic data, variables used for acid-base evaluation Glasgow Coma Score (GCS) and Acute Physiology and Chronic Health Evaluation II (APACHE II) score, average values and the results of logistic regression

Variables	Survivors (n = 68)	Nonsurvivors (n = 74)	*p*-value	OR (95% CI)
Age (years), $\bar{x} \pm SD$	56.43 ± 17.45	64.05 ± 15.77	0.009	1.028 (1.007–1.050)
Sex (male), n (%)	36 (53.7)	31 (46.3)	0.189	1.560 (0.804–3,029)
Hct (%), $\bar{x} \pm SD$	0.315 ± 0.06	0.310 ±0.07	0.649	0.321 (0.002–42.516)
Le ($n \times 10^9$/L), $\bar{x} \pm SD$	13.751 ± 5.76	13.689 ± 6.60	0.952	0.998 (0.947–1.053)
Na^+ (mmol/L), $\bar{x} \pm SD$	138.000 ± 5.32	142.35 ± 11.13	0.007	1.065 (1.017–1.114)
K^+ (mmol/L), $\bar{x} \pm SD$	3.824 ± 0.78	3.991 ± 0.82	0.221	1.297 (0.855–1,967)
PaO_2 (kPa), $\bar{x} \pm SD$	11.865 ± 2.81	11.699 ± 4.63	0.797	0.989 (0.908–1,077)
pH, $\bar{x} \pm SD$	7.374 ± 0.07	7.354 ± 0.12	0.259	0.152 (0.006–3.994)
SB (mmol/L), $\bar{x} \pm SD$	22.666 ± 4.82	22.046 ± 6.38	0.515	0.981 (0.925–1.040)
SBE mEg/L, $\bar{x} \pm SD$	1.235 ± 3.84	-0.153 ± 7.50	0.177	0.962 (0,909–1,018)
Lactates (mmol/L), $\bar{x} \pm SD$	1.732 ± 1.43	2.319 ± 2.49	0.106	1.171 (0.967–1.417)
Cl^- (mmol/L), $\bar{x} \pm SD$	101.540 ± 5.46	103.62 ± 6.44	0.043	1.060 (1.002–1.122)
Ca^{2+} (mmol/L), $\bar{x} \pm SD$	1.261 ± 0.370	1.225 ± 0.38	0.562	0.769 (0.316–1.870)
$PaCO_2$ (kPa), $\bar{x} \pm SD$	6.152 ± 1.61	5.835 ± 2.04	0.312	0.910 (0.759–1.092)
Albumin (g/L), $\bar{x} \pm SD$	27.88 ± 5.51	25.31 ± 6.54	0.015	0.932 (0.880–0.986)
SIDapp mEg/L, $\bar{x} \pm SD$	42.094 ± 7.60	44.566 ± 8.61	0.078	1.040 (0.996–1.085)
SIDeff mEg/L, $\bar{x} \pm SD$	34.568 ± 5.67	32.253 ± 8.35	0.061	0.956 (0.911–1.002)
SIG mEg/L, $\bar{x} \pm SD$	7.513 ± 7.15	12.352 ± 9.47	0.001	1.071 (1.027–1.116)
AG mEg/L, $\bar{x} \pm SD$	14.350 ± 6.86	17.427 ± 8.95	0.026	1.050 (1.006–1.095)
APACHE II, $\bar{x} \pm SD$	13.28 ± 6.10	18.93 ± 5.50	< 0.001	1.180 (1.103–1.262)
GCS, $\bar{x} \pm SD$	11.87 ± 3.42	10.88 ± 4.06	0.121	0.932 (0.853–1.019)

OR – odds ratio; CI – confidence interval; Hct – hematocrit; Le – leucocytes; MAP – mean arterial pressure; PaO_2 – partial pressure of oxygen; pH – Potential of hydrogen; SB – standard bicarbonates; SBE – standard base excess; $PaCO_2$ – partial pressure of carbon-dioxide; SIDapp – apparent strong ion difference; SIDeff – effective strong ion difference; SIG – strong ion gap; AG – anion gap; GCS – Glasgow Coma Score; APACHE II – Acute Physiology and Chronic Health Evaluation II.

Table 2

Receiver operating characteristics (ROC) curve analysis

Variables	Area under ROC curve	95% CI		*p* value	Cut-off value	Sn / Sp (%)
		Lower	Upper			
APACHE II	0.756	0.677	0.834	< 0.001	> 14	79.7/60.3
pH	0.496	0.400	0.591	0.927	<= 7.21	13.5/98.5
SB	0.483	0.387	0.578	0.724	<= 15.6	14.9/97.1
SBE	0.421	0.326	0.517	0.106	<= -3.3	32.4/91.2
Lactates	0.580	0.485	0.674	0.102	> 1.1	63.5/51.5
$PaCO_2$	0.426	0.332	0.521	0.131	<= 4.9	40.5/80.9
Albumin	0.370	0.279	0.462	0.008	<= 24	54.1/72.1
SIDapp	0.555	0.460	0.650	0.261	> 40.8	71.6/42.6
SIDeff	0.395	0.301	0.488	0.030	<= 31.8	54.0/69.1
SIG	0.651	0.561	0.742	0.002	> 10.6	56.7/73.5
AG	0.615	0.522	0.707	0.019	> 21	37.8/86.8

CI – confidence intervals; APACHE II – Acute Physiology and Chronic Health Evaluation II; SB – standard bicarbonates; SBE – standard base excess; $PaCO_2$ – partial pressure of carbon-dioxide; SIDapp – apparent strong ion difference; SIDeff – effective strong ion difference; SIG – strong ion gap; AG – anion gap; Sn – sensitivity; Sp – specificity.

Cl^-, albumin, SIDeff, SIG and AG. The largest area bellow the curve had: SIDeff, SIG, AG and APACHE II (Figure 1). It is important that *p* value is as far as possible from 0.05.

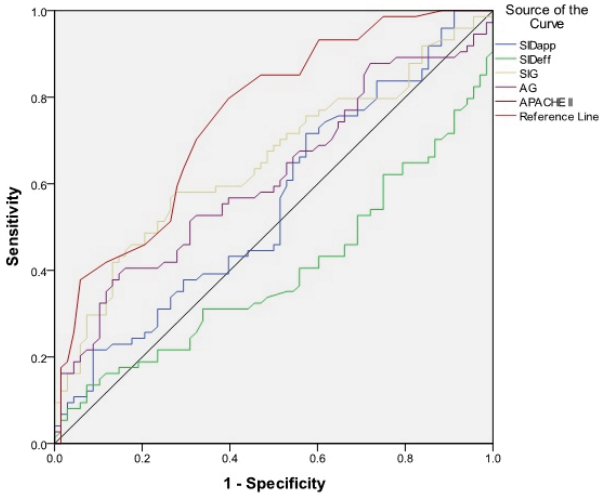

Fig. 1 – Receiver operating characteristic (ROC) curves of apparent strong ion difference (SIDapp), effective strong ion difference (SIDeff), strong ion gap (SIG), anion gap (AG) and Acute Physiology and Chronic Health Evaluation II (APACHE II) score.

Next, logistic models were made (Table 3). The first logistic model was a model to which predictors with $p < 0.1$ were added. The model was well-calibrated (H-L $p = 0.626$). This model indicated that the only significant predictor was the APACHE II score. However, we consider the model created with ENTER not adequate enough due to a disproportion between the sample size and the results of importance and the number of variables present in this model. Therefore, an additional model was created and predictors processed with the Forward method. It can be seen that at the first step APACHE II was a variable introduced as a statistically significant predictor with the least *p*-value. However, in this case the results of H-L test was $p = 0.072$, which indicated that APACHE II model was not enough for outcome prediction. In the step 2 SIG as a statistically significant predictor was added, and the result of HL test was $p = 0.274$. It indicated a well-calibrated model with good predictive capabilities.

Table 3

Results of logistic regression using the Enter and Forward method

Predictor	*p* value	OR	95% CI	
			Lower	Upper
Enter method				
Age	0.514	1.009	0.983	1.036
Na+	0.100	1.102	0.981	1.238
APACHE II	0.002	1.130	1.045	1.221
Cl^-	0.829	0.987	0.878	1.110
Albumin	0.174	0.950	0.882	1.023
SIDapp	0.550	0.503	0.053	4.802
SIDeff	0.593	1.850	0.194	17.658
SIG	0.525	2.080	0.218	19.870
AG	0.298	1.077	0.937	1.237
Forward method				
APACHE	< 0.001	1.180	1.103	1.262
APACHE II	0.000	1.171	1.093	1.255
SIG	0.012	1.062	1.013	1.114

OR – odds ratio; CI – confidence interval; SIDapp – apparent strong ion difference; SIDeff – effective strong ion difference; SIG – strong ion gap; AG – anion gap; APACHE II – Acute Physiology and Chronic Health Evaluation II.

Discussion

The present study show that the only reliable predictors of the 28-day survival rate in critically ill patients are SIG and APACHE II scores. It should be mentioned that this study is the first one focused on critically ill patients on mechanical ventilation exclusively, unlike the majority of similar studies [1, 5, 7, 12, 16]. Numerous studies have examined the predictive capability of standard and acid-base variables derived from the Stewart-Figge's quantitative biophysical method [1, 4, 12, 17, 18]. It has been noticed that the traditional acid-base variables (pH, AG, SB, and SBE) could be unsuccessful in complex acid-base disorders identification in critically ill patients.

If contradictory results from the literature on this phenomenon were taken into consideration, there would be unreliability regarding prognostic usefulness of some acid-base variables, and therefore their biological significance would be questionable. Although the severity of metabolic acid-base disorders or lactic acidosis in critically ill patients can predict the outcome of treatment, there are many inconsistencies regarding clinical relevance of these variables. Gunnerson et al. [1] analysed a possible discrepancy between SIG in healthy volunteers and stable patients before discharge from intensive care units. It was shown that stable patients at discharge had significantly higher levels of undetected anions comparing to healthy volunteers. This finding is explained with occult acid-base disorders, which cannot be identified by the standard metabolic status interpretation. The study conducted by Maciel and Park [4], shows that different anion proportions which cause

acidosis at admission to intensive care units are similar for survivors and nonsurvivors. At paediatric population of critically ill patients, Balasubramanyan et al. [5] have indicated that unmeasured anions can be used for lactate values prediction and that they predict mortality rate better than serum lactates. However, this discovery was contradictory to the study of Cusack et al. [16] conducted on adult population of critically ill patients. That study proved that initial pH and SBE had the best capability to predict treatment outcome among acid-base variables, while SIG had not significant prognostic power. On a narrow-selected patient population with serious vascular traumas, Kaplan et al. [19] found that SID/SIG methodology was a better 'tool' for estimation of potential mortality rate at patients than hypoperfusion markers and standard acid-base. Rocktaeschel et al. [14] in their study find that useful predictors of hyperlactatemia in adult general ICU patients are: BE, BEua (BE caused by unmeasured anions) and AG. Also, they find that in critically ill patients acid-base variables, calculated in four ways (AG, Agcorr-corrected anion gap, BEua, SIG), have a limited ability to predict hospital mortality. Therefore, they conclude that the nature, origin and true significance of unmeasured anions in critical illness remain unknown. Antonini et al. [20] conclude that despite the absence of acidaemia, progressive metabolic acidosis may be ongoing in the early phase of critical illness. However, metabolic acidosis determined by unmeasured anions is a clinically relevant phenomenon correlated with mortality.

In our study, ROC analysis indicates more potential predictors. It has been revealed by creation of logistic models with calibration methods [15], that outcome depends on SIG and APACHE II score. The arithmetic mean for SIG in the survivors group is significantly higher compared to the group of nonsurvivors. These values of SIG in the nonsurvivors group represent very large amount of undetected anions and indicate that organism is overloaded with acids. The results of this study support conclusions of other studies which claim that unmeasured anions, detected by the Stewart-Figge's methodology, identify a greater number of patients with acid-base disorders comparing to the conventional parameters (pH, AG, SB, SBE) [1, 4, 12, 18, 20, 21]. A great number of acidosis at critically ill patients were caused iatrogenically, by infusion solutions rich in chlorides and plasma expanders which act as weak acids [1, 8, 19]. However, unmeasured anions seem to represent heterogeneous set of various anions which is not always well-characterized because the anions come from many possible sources, and therefore future research should focus precisely on detecting their source. The scope of different diagnoses in this study is very heterogeneous, and it can be recommended that future studies on this phenomenon should focus on patients with clearly defined diagnosis (surgical, neurosurgical, neurological, internist etc.).

Conclusion

This study indicates that unmeasured anions if measured with quantitative biophysical method could have clinical implications, regarding not only the prognosis of critically ill treatment and its outcome, but also the early diagnostics of complex acid-base abnormality which cannot be detected with the traditional acid-base variables.

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Perceived parental acceptance/rejection, some family characteristics and conduct disorder in adolescents

Opažanje roditeljskog prihvatanja/odbacivanja, neke karakteristike porodice i poremećaj ponašanja adolescenata

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Abstract

Background/Aim. Conduct disorder is characterized by repetitive and persistent presence of dissocial, aggressive and defiant behavioral patterns, thus represents important public issue with comprehensive and far-reaching consequences both for the individual and society. The aim of this study was to investigate the differences in sociodemographic family characteristics and the prominence of parental acceptance/rejection dimensions in groups of adolescents with and without conduct disorder, as well as to examine the connection between parental acceptance/rejection dimensions and externalizing symptoms in the group of adolescents with conduct disorder. **Methods.** This research was conducted on 134 adolescents, aged 15 to 18, using the Parental Acceptance/Rejection Questionnaire (PARQ child), Youth Self-Report (YSR), and a questionnaire constructed for the purpose of this survey. **Results.** The results showed that the number of adolescents with conduct disorder coming from divorced families was significantly higher than from complete families (44.8% *vs* 13.4%, respectively; $p < 0.001$). Also, in this group of adolescents there was a statistically significantly higher number of parents suffering from psychiatric disorders compared to the controls (31.3% *vs* 8.9%; respectively; $p = 0.001$). The perceived rejection dimension and the total index of maternal acceptance/rejection were significantly higher in adolescents with conduct disorder than in those with no such disorder (132.30 ± 38.05 *vs* 93.91 ± 26.29 respectively; $p < 0.001$). Similar results were found for paternal acceptance/rejection dimension (129.40 ± 39.58 *vs* 86.10 ± 15.95 respectively; $p < 0.001$). Adolescents with conduct disorder and severe perceived maternal and paternal rejection showed a significantly higher average score on the subscale of externalizing symptoms (14.55 ± 4.45 and $13{,}27 \pm 5{,}05$) compared to adolescents with conduct disorder and lower total index of parental acceptance/rejection (8.32 ± 5.05 and 8.28 ± 5.08). **Conclusion.** The results suggest that adolescents with conduct disorder perceive their parents as more rejecting and less warm and supportive compared to adolescents without conduct disorder. The perception of significant and severe parental rejection was associated with a significantly higher averaged score on the subscale of externalizing symptoms in the group of adolescents with conduct disorder compared to those with no such disorder. It was found that adolescents with conduct disorder most often come from large families, have divorced parents or parents with multiple psychiatric disorders.

Key words:
conduct disorder; adolescent; family; risk factors; socioeconomic factors; questionnaires.

Apstrakt

Uvod/Cilj. Poremećaji ponašanja karakterišu se ponavljanim i trajnim disocijalnim, agresivnim i devijantnim ponašanjem, pa tako predstavljaju važan društveni problem sa sveobuhvatnim i dalekosežnim posledicama za pojedince i društvo. Cilj ovog rada bio je da se ispitaju razlika u sociodemografskim karakteristikama porodica adolescenata i izraženosti dimenzija roditeljskog prihvatanja/odbacivanja između grupa adolescenata sa i bez poremećaja ponašanja, kao i ispitivanje povezanosti dimenzija roditeljskog prihvatanja/odbacivanja sa eksternalizacionim simptomima u grupi adolescenata sa poremećajem ponašanja. **Metode.** Ispitivanje je obuhvatilo 134 adolescenta, starosti od 15 do 18 godina. Primenjeni su: Upitnik roditeljskog prihvatanja/odbacivanja (*Parental Acceptance/ Rejection Questionnaire*, PARQ *child*), Upitnik za samoprocenu mladih od 11 do 18 godina (*Youth Self-Report*, YSR), kao i opšti upitnik sačinjen za potrebe ovog istraživanja. **Rezultati**. U grupi sa poremećajem ponašanja statistički značajno više adolescenata potiče iz razvedenih porodica u odnosu na kompletne porodice (44,8% *vs* 13,4%; $p < 0{,}001$), a u istoj grupi statistički je značajno više roditelja sa psihičkim bolestima (31,3% *vs* 8,9%; $p = 0.001$) u odnosu na grupu adolescenata bez poremećaja ponašanja. Dimenzije percipiranog odbacivanja kao i totalni indeks prihvatanja/odbacivanja za majku su statistički značajno veće u grupi adolescenata sa po-

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remećajem ponašanja u odnosu na one bez poremećaja ponašanja (132,30 ± 38,05 *vs* 93,91 ± 26,29; $p < 0.001$). Slični rezultati dobijeni su i za dimenzije prihvatanja/odbacivanja za oca (129,40 ± 39,58 *vs* 86,10 ± 15,95; $p < 0.001$). Adolescenti sa poremećajem ponašanja i ozbiljnim percipiranim odbacivanjem majke i oca pokazuju znatno veći prosečni rezultat na supskali eksternalizacionih simptoma (14,55 ± 4,45 and 13,27 ± 5,05) u odnosu na adolescente sa poremećajem ponašanja i nižim totalnim indeksom prihvatanja/odbacivanja za oba roditelja (8,32 ± 5,05 and 8,28 ± 5,08). **Zaključak.** Rezultati istraživanja ukazuju da adolescenti sa poremećajem ponašanja percipiraju svoje roditelje kao više odbacujuće i manje tople i podržavajuće u odnosu na adolescente bez poremećaja ponašanja. Percepcija značajnog i ozbiljnog odbacivanja od strane roditelja bila je povezana sa višim prosečnim skorom eksternalizacionih simptoma u grupi adolescenata sa poremećajem ponašanja. Nađeno je da adolescenti sa poremećajem ponašanja dolaze iz porodica koje karakteriše mnogočlanost, učestali razvodi roditelja i više psihijatrijskih oboljenja kod roditelja.

Ključne reči:
ponašanje, poremećaji; adolescent; porodica; faktori rizika; socioekonomski faktori; upitnici.

Introduction

According to ICD-10, conduct disorder is characterized by repetitive and persistent presence of dissocial, aggressive and defiant behavioral patterns [1]. Such behavior, when at its most extreme for the individual, should amount to major violations of age-appropriate social expectations, and is therefore more severe than ordinary childish mischief or adolescent rebelliousness. The diagnosis is based on the following behavior examples: excessive fights and bullying, cruelty to people and animals, severe destructiveness to property, arson, theft, repeated lying, truancy from school and running away from home, unusually frequent and severe temper tantrums, defiant, provocative behavior and persistent severe disobedience. All these forms of behavior, if prominent, may be sufficient for diagnosis only if they persist over a period of time (minimum of 6 months) [1].

In relation to the severity of the disorder and according to current classification systems, conduct disorder is graded as mild, moderate and severe [2]. This classification is important both for diagnostic and psychosocial interventions because, theoretically speaking, it is possible that a child who lies, runs away from home and skips school has the same diagnosis as a child who has robbed a bank with a gun or raped someone. In relation to the onset of conduct disorder symptoms there are two subgrups: childhood-onset group and adolescent-onset group [2]. Children in childhood-onset group often begin showing severe conduct problems in childhood as opposed to those whose onset of severe antisocial behavior coincides with the onset of pyberty. Moffitt [3] and Moffitt and Caspi [4] has proposed that problem behavior in childhood-onset group is developed through a transactional process involving a difficult and vulnerabile child (impulsive, with verbal deficit, attention deficit disorder and hyperactivity or difficult temperament) who experiences an inadequate rearing environment (severe family dysfunction, parental antisocial behavior, poor parental supervision, poor quality schools).

In contrast, children in the adolescent-onset group engage in antisocial and delinquent behaviors as a misguided attempt to obtain a subjective sense of maturity and adult status in a way that is maladaptive (e.g. breaking societal norms) but encouraged by an antisocial peer group [3, 4]. However, these adolescents may still have impairments that persist into adulthood due to the consequences of their adolescent antisocial behavior (e.g. criminal record, dropping out of school, substance abuse) [4].

Conduct disorder represents important public issue with comprehensive and far-reaching consequences both for the individual and society. The most recent prospective longitudinal Cambridge Study in Delinquent Development reports that boys with dissocial behaviour aged 8–10 exhibit the same pattern of behavior at the age of 14, and 43% of them show the same behavior at the age of 18 [5]. Some studies suggest that about 50% of children with conduct disorders develop dissocial personality disorder in adulthood [6], and are at risk of developing a wide range of other maladaptive outcomes, including substance abuse, termination of education, mental disorders [7], prison sentences, work and family problems and physical health deterioration manifested in a higher injury rate, hospitalization, sexually transmitted diseases, smoking and chronic respiratory diseases, and violent death [8].

Risk factors for the development of conduct disorders are classified as personal, family or environmental (relating to peers, school and wider community). In the context of family risk factors, studies suggest that inadequate parenting, expressed through tough and inconsistent parental discipline, poor parental monitoring and supervision, low levels of positive parental involvement and parental rejection, is significantly associated with externalizing behavior of children and adolescents [9, 10]. Other factors in the etiology of child behavior problems include family conflict, the number of parents present, family size, socioeconomic status, criminality in parents, parental psychiatric disorder, child abuse [11–13]. Nevertheless, even after controling these factors, parental rejection continues to be significantly associated with behavior problems [13].

Parental Acceptance/Rejection Theory (PARTheory) by Rohner et al. [14] emphasizes the impact of parental rejecting and accepting behavior on child's behavioral, cognitive and emotional development. Parental acceptance and rejection refers to the emotional and affective relationship between parents and children, and the physical, verbal and symbolic behaviors parents use to express their feelings for their children.

Parental acceptance and rejection together form a "warm" dimension of the upbringing approach designed as a bipolar dimension. At one pole there is parental acceptance

relating to warmth, affection, care, support and, in general, love that a child may experience in relationship with parents or careers. At the other pole there is rejection and lack of parental warmth and emotionality, which may be perceived as any combination of four basic rejection expressions: parents' physical or verbal hostility, indifference or neglect, and undifferentiated parental rejection. Hostility includes a range of emotions from objection and disapproval to anger, reservation and resentment, while indifference implies a lack of concern and affection for the child. Undifferentiated rejection represents such kind of rejection due to which the child feels unaccepted without clear perception of aggression and neglect by parents.

Cross-cultural studies indicate that unipolar depression, depressive affect, behavioral problems including conduct disorder, externalizing symptoms, delinquency and substance abuse are universal correlates of parental acceptance/rejection regardless of cultural, gender, racial and socioeconomic differences [13].

The aim of the study was to examine some characteristics of the family (structure, size, parental disorders) in groups of adolescents with and without conduct disorder, to investigate perceived parental acceptance/rejection in groups with and without conduct disorder, to investigate the relationship between perceived parental acceptance/rejection and externalizing symptoms in the group with conduct disorder.

Methods

The study was conducted at the Department of Children and Adolescent Psychiatry, Mental Health Clinic, Clinical Center Niš, Serbia in 2011/2012. It included 134 adolescents, aged 15 to 18. The examined group consisted of 67 outpatient or hospitalized adolescents, with conduct disorders. The diagnosis of conduct disorder was based on clinical interviews and existing criteria for conduct disorder [1]. The subjects with the following comorbid diagnoses were excluded from the study: attention deficit disorder and activity disorder, mental insufficiency under 80 on the basis of standard psychological tests, acute psychotic disorder and drug addiction. The group without conduct disorder (the control group) consisted of 67 high school students. Both groups were matched for sex, age and place of residence. Subjects and parents/caregivers gave informed consent to participate in research.

Questionnaire designed for study purposes consisted of questions relating to sociodemographic features of examinees: gender, age, the number of household members, marital status of parents, and the presence of parental mental illness. The questionnaire was filled out by the researcher based on interviews with adolescents and parents and data from the medical records or polyclinic records.

Parental Acceptance-Rejection Questionnaire Child Version (Child PARQ) [14] is a self-report questionnaire designed to measure individual perceptions of parental acceptance/rejection. The questionnaire contained four subscales which measured four dimensions of parenting: parental warmth/acceptance (W/A), parental hostility/aggression (H/A), parental indifference/neglect (I/N), parental undifferentiated rejection (U/R). Each questionnaire statement contained a description of parental behavior. The examinees were asked to choose one of the answers on the Likert scale ranked from 1 (almost never true) to 4 (almost always true), depending on the extent to which they agree or disagree with the given statement related to parental behavior. The result of each examinee can be expressed on individual subscale and as a total PARQ (sum of all four scales, with the entire warmth scale reverse scored). The total score ranges from 60 to 240, whereby results equal to or greater than 150 indicate a perception of significant and severe parental rejection.

The Youth Self-Report (YSR)[15] is a scale of emotional problems and behavior problems. The questionnaire has two parts: competence scale and the scale of problems with 112 items, which are grouped into eight syndrome scales. The seventh and eighth scale referred to the group of externalizing problems – aggressive behavior (behavior aimed at drawing attention, passive aggressive and open aggressive behavior), and rule breaking behavior (morality aspect, violation of the legal norms, socially immature and maladapted behavior) that represent symptoms of behavioral disorders. The examinees were supposed to assess the extent to which they could relate to a particular problem on the Likert scale. Responses ranged from 0 (not true) to 2 (completely true). The results of the study were statistically analyzed on the scales in relation to the study objective (the sum of scores on the seventh and eight syndrome scales).

Comparisons between groups were made by *t*-test, Mann-Whitney test or χ^2-test. A p value < 0.05 was considered statistically significant. Statistical analyses were done with SPSS 16.0 for Windows.

Results

Sociodemographic characteristics of adolescents with and without conduct disorders are shown in Table 1. There

Table 1

Sociodemographic characteristics of the adolescents with and without conduct disorders

Parameters	With conduct disorder	Without conduct disorder	*p*
Age (years), $\bar{x} \pm SD$	17.15 ± 0.97	17.19 ± 0.68	
Gender (M/F), n	30/37	28/39	0.673
The number of children in the family, n			
1	13	10	0.008
2	33	50	
> 2	21	8	
Divorced parents, n	30	9	< 0.001
Parental psychiatric disorders, n	21	6	0.001

M/F – male/female.

was no significant difference in age in the groups of adolescents with conduct disorder compared to the control group. Statistically significant difference was found referring to the number of children in the examined groups ($p = 0.008$). In the group of subjects with conduct disorder there was statistically significant number of adolescents coming from divorced families compared to controls: 44.8% *vs* 13.4% ($p < 0.001$). Also, the number of parents suffering from psychiatric disorders was found to be significantly higher in the adolescents with conduct disorder compared to controls: 31.3% *vs* 8.9% ($p = 0.001$).

The YSR questionnaire showed that adolescents with conduct disorder had a significantly higher averaged score on the subscale of externalizing problems (12.43 ± 4.66) compared to the control group (5.40 ± 3.46; $p < 0.001$).

The results showed a statistically significant difference between the two examined groups in all dimensions of perceived parental acceptance/rejection relating to both father and mother (Table 2). Dimensions of maternal warmth/acceptance were significantly higher in the subjects without symptoms compared to those with conduct disorder. The other three dimensions of perceived rejection (H/A, I/N, U/R) and the total index of maternal acceptance/rejection were significantly higher in the patients with conduct disorder (Figure 1). The H/A dimension had the highest score, I/N had lower score, and U/R dimension had the lowest score.

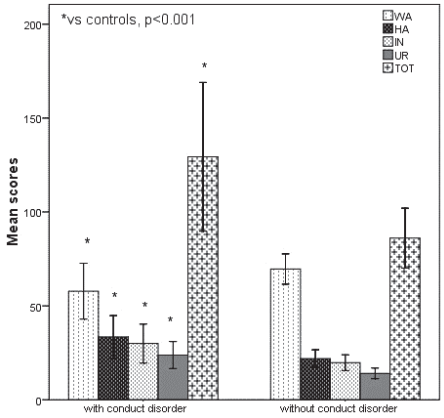

Fig. 1 – Mean values of perceived maternal acceptance/rejection in the adolescents with and without conduct disorder.

W/A – parental warmth/acceptance; H/A – parental hostility/aggression; I/N – parental indifference/neglect; U/R – parental undifferentiated rejection; TOT – total Parental Acceptance/Rejection Questionnaire score.

Analysis of the questionnaire scores of paternal acceptance/rejection showed that the dimension of perceived paternal warmth (W/A) was significantly higher in the subjects without symptoms compared to those with conduct disorder. The other three dimensions of perceived paternal rejection (H/A, I/N, U/R) were significantly higher among the subjects with conduct disorder, as well as a total index of parental acceptance/rejection (Figure 2). Scores were lower for fathers than mothers: H/A dimension had the highest score, I/N dimension had lower score, U/R dimension had the lowest score.

In 20 of the patients (29.85%) with conduct disorder the total index of maternal acceptance/rejection was above 150, which indicated serious and significant perceived maternal rejection. Twenty six subjects (38.81%) from the same group had the total index of paternal acceptance/rejection above 150.

The adolescents with conduct disorder and serious perceived maternal rejection (total index of maternal acceptance/rejection above 150) showed a significantly higher average score on the subscale of externalizing symptoms compared to adolescents with conduct disorder and lower total index of maternal acceptance/rejection. The analysis of the results of paternal acceptance/rejection and externalizing symptoms showed that the average value on the subscale of rule breaking behavior was significantly higher in the ado-

Table 2

Acceptance-rejection dimensions for the mother and the father of adolescents with and without conduct disorders

Acceptance-rejection dimensions	Mother ($\bar{x}$ ± SD)			Father ($\bar{x}$ ± SD)		
	with conduct disorder	without conduct disorder	*p*	with conduct disorder	without conduct disorder	*p*
W/A	54.43 ± 15.19	65.22 ± 11.26	< 0.001	57.82 ± 14.86	69.65 ± 8.04	< 0.001
H/A	32.36 ± 11.77	22.22 ± 7.63	< 0.001	33.46 ± 11.34	21.93 ± 4.66	< 0.001
I/N	31.55 ± 9.21	22.37 ± 6.96	< 0.001	29.97 ± 10.37	19.75 ± 4.20	< 0.001
U/R	22.82 ± 7.64	14.54 ± 4.28	< 0.001	23.79 ± 7.21	14.07 ± 2.84	< 0.001
Total	132.30 ± 38.05	93.91 ± 26.29	< 0.001	129.40 ± 39.58	86.10 ± 15.95	< 0.001

W/A – parental warmth/acceptance; H/A – parental hostility/aggression; I/N – parental indifference/neglect; U/R – parental undifferentiated rejection; Total – total Parental Acceptance/Rejection Questionnaire score.

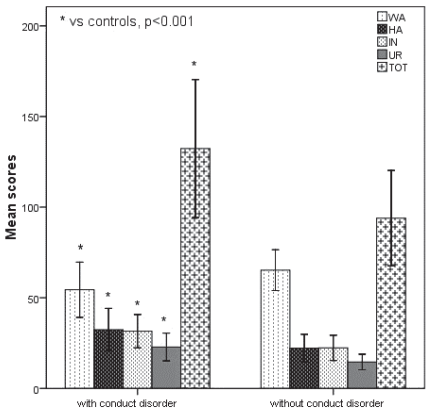

Fig. 2 – Mean values of perceived paternal acceptance/rejection in the adolescents with and without conduct disorder.

W/A – parental warmth/acceptance; H/A – parental hostility/aggression; I/N – parental indifference/neglect; U/R – parental undifferentiated rejection; TOT – total Parental Acceptance/Rejection Questionnaire score.

lescents with conduct disorder and perception of severe paternal rejection (a total index of paternal acceptance/rejection was above 150) (Table 3).

Table 3

Externalizing symptoms in the adolescents whose total PARQ score for the mother and the father is less than or above 150

Externalizing symptoms	Mother ($\bar{x} \pm SD$)		Father ($\bar{x} \pm SD$)		p
	total PARQ less than 150	total PARQ above 150	total PARQ less than 150	total PARQ above 150	
Rule breaking behavior	2.69 ± 1.95	5.15 ± 1.69	2.68 ± 1.99	4.62 ± 1.81	< 0.001
Aggresive behavior	5.63 ± 3.51	9.40 ± 3.25	5.60 ± 3.49	8.65 ± 3.64	< 0.001
Total	8.32 ± 5.05	14.55 ± 4.45	8.28 ± 5.08	13.27 ± 5.05	< 0.001

PARQ – Parental Acceptance/Rejection Questionnaire.

The results showed statistically significant differences in scores for rule-breaking behavior ($p = 0.030$) in the adolescents with total PARQ score above 150 for both mothers and fathers. There were no statistically significant differences in scores for the other two parameters (Table 4).

adolescents with conduct disorder compared to the control group. Perceived acceptance and rejection enables individuals to interpret parental behavior through their own cultural and individual filters, thus avoiding the possibility of misinterpretating the meaning of parental behavior. Although adolescents' reports and their response to perceived parental behavior most likely involve some permanent and momentary characteristics of the respondents, it also relies on how they experience and remember their parents' behavior, which is indicative of the model of parental behavior to which they are exposed [14].

Table 4

Externalizing symptoms in the adolescents whose total PARQ score for both mother and father is above 150

Externalizing symptoms	PARQ score lower than 150 ($\bar{x} \pm SD$)	PARQ score above 150 ($\bar{x} \pm SD$)	p
Rule-breaking behavior	4.32 ± 1.51	5.54 ± 1.81	0.030
Aggressive behavior	8.38 ± 3.33	9.09 ± 4.08	0.805
Externalizing	12.61 ± 4.35	14.64 ± 5.50	0.324

PARQ – Parental Acceptance/Rejection Questionnaire.

Discussion

The largest number of studies indicated that broken families and divorce significantly increased the risk of developing emotional and behavioral problems [11]. In our study 44.8% of the subjects with conduct disorders had divorced parents. It was highlighted that the risk factors for such disorders included not only the very act of divorce, separation or the establishment of new family but also the context of divorce and separation as well: poor communication, conflict, and physical altercations, triangulation of children, parental anxiety and stress, poor financial conditions and adaptation to new partners.

A greater number of adolescents with conduct disorder live in large families, which is in accordance with other studies indicating that big families represent a risk factor for the development of conduct disorder [16].

Parental psychopathology was more frequent in the subjects with conduct disorder. It was the parental dissocial behavior (parental criminality, alcohol and substance addiction) and maternal depression that represented a significant predictor of behavioral disorders in childhood and adolescence [17, 18]. This could be explained by the intergenerational continuity of exposure to multiple risk factors, the mediation of environmental factors (eg, poor monitoring of children) and/or genetic transmission mechanisms of aggressive behavior [17].

The results of our study show a statistically significant difference in perceived parental acceptance/rejection among

The adolescents with conduct disorder perceived their mother more often as hostile, aggressive (physical, verbal or non-verbal aggressive gestures) and discarding. Our results were consistent with the results found in other studies [13, 19].

On the other hand, the role of the father in upbringing of a child may represent support to mother or important factor affecting the development and socialization of children, boys, in particular. In our study, subjects with conduct disorder perceived behavior of their fathers as more rejecting compared to the control group. The highest average value was obtained on the subscale of perceived paternal aggression/hostility that was, however, lower than the perceived maternal aggression.

Studies on the connection between parental rejection and behavioral disorders of children report that the contribution of parents and children in the development of conduct disorder is equal[13]. Parental rejection leads to children's hostile and aggressive behavior, and if such behavior continues parents show less warmth and support to them. Regardless of this reciprocal relationship, researchers wanted to know whether it was possible to determine the dominant direction of causality. It turned out that parental rejection preceded the development of conduct disorder [13].

The way in which hostile and aggressive parents encourage aggressiveness in children is explained through a number of theoretical models: identification with aggres-

sor [20], model learning [21], or imitating the one "who has the power" [22]. This leads to the formation of relationships that causes and supports violence and to the adoption of elements of parental distorted and violent style as legitimate ways of interaction between people. Therefore, it is believed that the aggressive behavior adopted in early childhood remains relatively stable throughout the whole life [23].

The perception of serious and significant parental rejection proved to be associated with larger self-assessed values of externalizing symptoms in the group of subjects with conduct disorder. The expressed perceived paternal rejection was associated with higher mean values on the subscale of rule violations. Our finding is consistent with the findings of other authors [13, 19, 24] who state that the low level of perceived parental warmth and high levels of perceived parental rejection are associated with prominent externalizing symptoms in children. The observed relationship may be interpreted within the specific development of those individuals who perceive themselves seriously and significantly rejected by their parents or other affectionate figures. They develop specific personal disposition expressed in terms of hostility, aggression, emotional coldness, low self-esteem and emotional instability, negative views of themselves and tend to perceive life events and reactions of other people in the negative and hostile way [14]. Theoretically, these personal dispositions are expected to be based on expressed aggression and violations of legal norms, socially maladapted and immature behavior. A recent research suggests that young people with conduct disorder and callous-unemotional interpersonal trait (lack of empathy, egocentrism, superficial charm, and rejecting guilt and remorse) form a special subgroup that is characterized by persistent and severe models of aggressive and delinquent behavior and higher instrumental aggression [25–27]. Etiological trajectory traits of callousness/unemotionality are the subject of numerous studies. Some studies report that parental rejection, particularly serious perceived maternal rejection, is a significant predictor of callous/unemotional trait [28, 29]. Pardini et al. [29] examined a connection between parental emotional warmth and callous/unemotional trait in children 9 to 12 years of age who expressed moderate and severe aggression. The children who perceived their parents as warm and "involved" in the upbringing tended to decrease the expression of intrapersonal traits and dissocial behavior in general. The same authors concluded that the quality of children's "inner" concept of parent-child relationship was an essential precursor of callousness/unemotionality in childhood.

Effective parenting can be a powerful protective factor that surpasses other family, school or community risk factors. Therefore, it is not surprising that nowadays there is a growing number of training programs for the development and improvement of parenting skills and the promotion of positive parenting.

This study has several limitations: it is based on a relatively small sample of respondents and their self-assessment and conclusions relating to the parental influence on a child neglecting individual and gender differences among adolescents that may be important determinants of parental behavior as well. However, having in mind the specificity of this problem, it is emphasized that respondent's subjective experience is very important for the study of parental acceptance/rejection.

Conclusion

There are significant differences in the perceived parental acceptance/rejection between the group of adolescents with conduct disorder and the control group. The adolescents with conduct disorder came from large families or families with higher incidence of parental divorce and parents with psychiatric disorders. They significantly perceive their parents as more aggressive, neglecting and rejecting compared to adolescents without conduct disorder. Parental rejection was associated with higher self-assessed values on the subscale of externalizing symptoms in the group of adolescents with conduct disorder. Further research in the field of parenting and conduct disorders may enable better understanding of parental risk and protective factors in the development of disorders, as well as the development of prevention and treatment programs for adolescents with conduct disorder and their parents.

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Knowledge, attitudes and behavior of children in relation to oral health

Informisanost, stavovi i ponašanje djece prema oralnom zdravlju

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Abstract

Background/Aim. Health education plays a very important role in maintaining health of individuals. Good oral health, as a part of general health, is largely dependent on the level of knowledge, attitudes and habits that children already have. The aim of this study is to examine the level of knowledge and habits in children regarding oral hygiene, diet and bad habits. **Methods.** The study included 506 school children aged 12 and 15 years in three towns (Foča, Čajniče, Kalinovik, Bosnia and Herzegovina). The survey was conducted in order to assess knowledge, attitudes and habits that children have in relation to their own oral health. **Results.** Most respondents stated that they began to brush their teeth at the age of 4, while a smaller number linked beginning of tooth brushing to the start of school. The parents more often help the boys during tooth brushing. A total of 54.9% of children brush their teeth after every meal, while 40.1% of them brush teeth only once during the day. Twelve year olds brush their teeth more often, especially after a meal. A total of 92.5% of children had never used fluoride tablets nor are the tablets recommended to them by anyone. More than half of the children (61.7%) visited the dentist for the first time before starting school that is on the regular examination that is performed upon enrollment to school. A pain as a reason for dental visits was present in 43.9%, while the preventive check in only 31.4% of the children. **Conclusion.** Children included in this study, particularly 15-year-olds, are quite well informed about teeth brushing frequency and proper selection of tools for hygiene maintenance, but this knowledge is not applied. Girls are more responsible for their own health, and come regularly to the preventive dental checkups.

Key words:
child; adolescent; oral health; attitude to health.

Apstrakt

Uvod/Cilj. Zdravstveno vaspitanje ima izuzetno važnu ulogu u očuvanju zdravlja pojedinca. Dobro oralno zdravlje, kao deo opšteg zdravlja, umnogome zavisi od stepena informisanosti, stavova kao i navika koje djeca već imaju. Cilj rada bio je da se ispita stepen informisanosti i navike djece prema oralnoj higijeni, način ishrane i loše navike. **Metode.** U istraživanje je bilo uključeno 506 školske djece uzrasta od 12 i 15 godina iz tri grada (Foča, Čajniče, Kalinovik). Za ocjenu informisanosti, kao i stavova i navika koje djeca imaju prema sopstvenom oralnom zdravlju, sprovedena je anketa. **Rezultati.** Većina ispitanika je navela da je počela da pere zube sa navršene četiri godine, dok manji broj svoj početak pranja zuba vezuje za polazak u školu. Roditelji češće pomažu dječacima prilikom pranja zuba. Ukupno 54,9% djece pere zube nakon svakog jela, dok 40,1% djece samo jednom u toku dana pere zube. Dvanaestogodišnjaci češće peru zube i to nakon obroka. Ukupno 92,5% djece nije nikada upotrebljavalo fluor tablete, niti im ih je neko preporučivao. Veći broj djece (61,7%) prvi put je posjetilo stomatologa pred polazak u školu, tj. na redovnom sistematskom pregledu koji se obavlja pri upisu u školu. Bol kao razlog posjete stomatologu bila je zastupljena kod 43,9%, dok preventivna kontrola samo kod 31,4% djece. **Zaključak.** Djeca uključena u ovo ispitivanje, a posebno petnaestogodišnjaci, dosta su dobro informisana o redovnosti održavanja oralne higijene i pravilnom izboru pribora, ali to znanje ne primjenjuju. Djevojčice su odgovornije prema sopstvenom zdravlju jer dolaze redovnije na preventivne stomatološke preglede.

Ključne reči:
deca; adolescenti; usta, zdravlje; stav prema zdravlju.

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Introduction

The occurrence of two most common diseases of the oral cavity, dental caries and periodontal disease, as well as reduction in their distribution significantly depend on the knowledge about their origin and measures for their prevention. Health education plays a role in promotion of the right information in order to prevent these diseases. Health education aims to develop responsibility in every individual for their own health, health of the nearest environment as well as for communities where we live and work [1]. In the domain of dental care, health education task is to inform and motivate individuals and society in total to preserve the health of mouth and teeth. The task is also to promote the establishment of regular and proper oral hygiene habits, establishment of proper nutrition, as well as the use of fluoride.

Oral health is largely dependent on habits, attitudes and behaviors that are current hygienic-dietary habits in the family [2]. The parents, as the highest authority, have a crucial importance in forming the personality of the child with the positive attitude to oral health by their health education influence on children [3]. Studies have shown that children, who visit the dentist more often, are more informed about teeth and mouth health. However, implementation of existing knowledge greatly depends on motivation of patient (possible children) to preserve one's own health, but also on the motivation and commitment of parents [4].

Health education program cannot be based only on one-off provision of information, because it usually gives poor results and short-term effects. The program should be based on active learning process and education, both in preschool and school children, their parents and the whole community [5]. The role of dentists is essential in children and parents counseling, as well as in finding an adequate way for the implementation of preventive measures.

Recent researches in the Eastern Europe countries show that the oral health of school children must be improved and school health education programs are needed for health promotion. The engagement of parents and teachers in these programs is essential [6].

Various literature data indicate that the most efficient and economically most cost-effective method of oral preventive program worldwide is the School Dental Care (SDC) [7]. It is particularly significant because schools may include those children who cannot come (due to socioeconomic reasons), do not want to come (because of fear) or who are not sufficiently motivated and interested in coming for regular checkups and dental repairs [8].

Research on oral hygiene habits showed that there are two widely defined forms of behavior – self-defense (oral hygiene, decreased intake of refined carbohydrates, fluoride use), and dental services usage (health education, regular dental checkups and professionally applied preventive measures) [8]. Habits of regular and proper oral hygiene accepted during childhood are extremely important for the preservation of health of teeth and mouth through life. Improvement of oral-hygiene habits led to enhanced dental health in the elderly population [8].

The aim of this study was to evaluate the level of knowledge, current attitudes and habits that children have in relation to oral hygiene maintenance, diet and the presence of the bad habits.

Methods

The study included 506 children: 324 aged 12 years and 182 aged 15 years in three towns (Foča, Čajniče, and Kalinovik, Bosnia and Herzegovina). The sample comprised approximately the same number of boys (263, 51.97%) and girls (243, 48.03%). To assess knowledge, attitudes and habits that children have in relation to their own oral health, survey was conducted using a specially prepared questionnaire (Addendum 1).

The survey consisted of 23 questions in total, divided into three parts. The first part of the questions included questions that examined children's knowledge about proper oral hygiene and the reasons for dental visits. In addition to the data on knowledge part of the questions was related to their hygiene habits.

The second part of the survey contained the history data related to condition of the gums in the sense of the existence of subjective symptoms such as of bleeding, swelling, pain, unpleasant odor. These data indicated the absence or presence of weaker or stronger gingival inflammation.

The third group of questions was related to bad habits, the presence or absence of bad habits as well as the type of food they like to consume.

In order to present and interpret data obtained from this survey in easier and more simple manner during the statistical analysis, within some questions there were answers grouped in response to the accuracy or impact on the incidence of tooth decay (with each such question there will be note with the symbol *).

One of such questions was the question from the first group of questions related to the knowledge of patients, in order to establish the means used for oral hygiene maintenance. In order to facilitate statistical analysis, the answers were deployed in three groups according to their accuracy. The accurate answers were considered the circled responses: toothbrush, toothpaste with fluoride, dental floss. As partially correct were considered the answers when the respondents circled only two responses from the aforementioned three. Incorrect were the responses of all other offered combinations.

Another such question was one in the third group of questions related to examination of the presence of bad habits. In addition to the knowledge of the respondents about the importance of nutrition to caries or periodontal disease occurrence, it was important to know about their favorite food. There were four responses. In order to simplify statistical analysis, grouping of responses was performed. The answer was: cariogenic food, if the respondents rounded up chocolate, coca-cola, fruit; conditionally cariogenic if rounded fruit; non-cariogenic if they rounded up vegetables.

The study began by the prearranged order after obtaining the approval of Medical Faculty in Foča Ethic Commit-

tee, consent of school directors, written consent from parents/guardians. The study was conducted according to the "Principles of good research practice" and the Declaration of Helsinki for Medical Research.

Prior to distribution of the questionnaires, the children were given instructions on how to fill the questionnaire and unfamiliar terms were explained to them. For each question, they had to circle one or more answers.

Data obtained in this study were processed by SPSS program version 11.5 (SPSS Inc., Chicago, IL, USA). The results of the survey were analyzed using the χ^2 test and values of $p < 0.05$ were considered statistically significant.

Results

The research included an approximately equal number of boys (51.97%) and girls (48.03%), 64.03% twelve year olds and 35.97% fifteen year olds, respectively. By means of questionnaire data related to the level of oral hygiene knowledge, reasons for dental visits of children involved in this study, their hygiene and dietary habits, the existence of local risk factors for periodontal disease, as well as to the influence of the parents on children's oral health, were obtained. Analyzing the questionnaire, which consisted of three groups of questions, a statistical significance was observed in the first and the third group.

Almost all the respondents considered the proper and regular oral hygiene as important for dental health, while only 16.4% thought dental hygiene essential to have clean teeth. Most respondents stated to have started teeth brushing at the age of 4, while a smaller number of them associated teeth brushing with enrollment to school. The parents more often help boys with tooth brushing as shown by a statistical significance ($p < 0.01$). More than half of examined children brush their teeth after every meal, while a smaller proportion of children brush teeth only once during the day. Twelve year olds brush teeth more often and after every meal, while fifteen year olds brush only once during the day ($p < 0.05$).

Fifteen year olds were better informed about proper frequency of tooth brushing compared to twelve year olds, while 18.2% of twelve year olds considered brushing twice a day enough ($p < 0.01$) (Figure 1). Girls were more frequent in brushing their teeth and mouth, while boys did so only occasionally ($p < 0.01$) (Figure 2). Fifteen year olds brushed their teeth longer than the twelve year olds ($p < 0.01$).

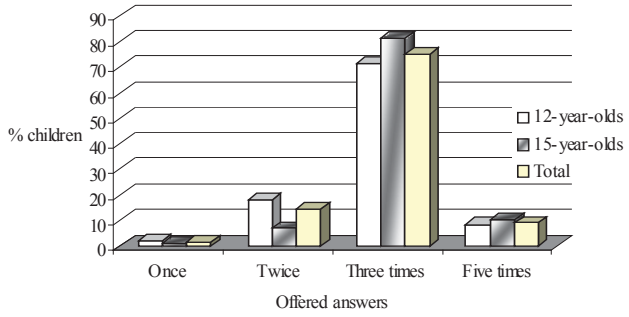

Fig. 1 - Distribution of answers to the question: How many times teeth need to be washed a day?

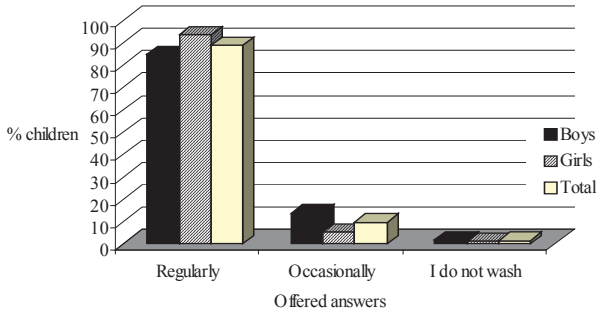

Fig. 2 – Distribution of answers to the question: Do you wash your teeth and mouth regularly?

Half of the examined children gave partially correct answer to question number eight (tooth brush and tooth paste) (Figure 3), while a smaller number of them circled some of the remaining two combinations. A third knew and circled the correct answer. However, fifteen year olds better knew what tools should be used for daily maintenance of proper oral hygiene, compared to the younger population of students ($p < 0.01$). Boys were more likely to avoid brushing teeth than girls ($p < 0.01$).

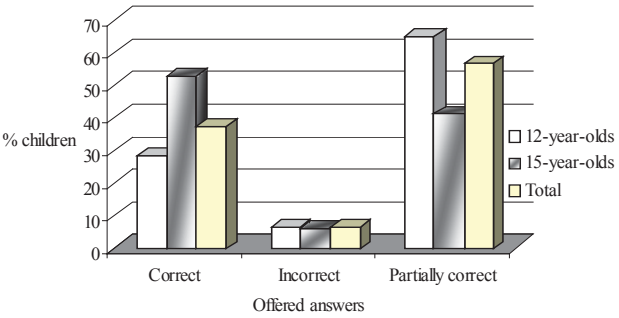

Fig. 3 – Distribution of answers* to the question: What means do you use for oral hygiene maintenance?

*** Answers grouped according to accuracy, due to stastitical analysis.**

The disturbing fact was that 92.5% of children had never used fluoride tablets nor have ever been recommended by anyone, those children did not know what it was fluoride or what was its purpose.

More than half of children (61.7%) visited the dentist for the first time just before starting school, at the regular medical examination performed upon enrollment to school. Slightly more girls visited the dentist for the first time at the age of 3 and 7 years of life, while the boys met with the dentist for the first time at the age of 10 ($p < 0.05$). Toothache as a reason for visiting the dentist was present in the highest percentage of boys of all ages while girls more often went on preventive examinations, with the respect to the schedule at the dentist ($p < 0.01$) (Figure 4).

History data explaining gum health were included in the second set of questions (Addendum 1). The responses also showed if there were any subjective discomforts, swellings, painful gums with the tendency to bleeding or unpleasant odor. This section did not reveal any differences by gender or by age.

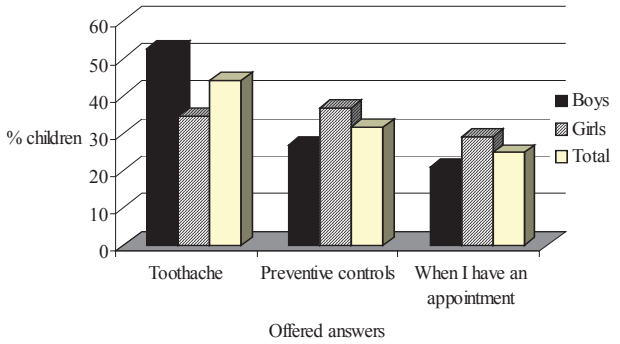

Fig. 4 – Distribution of answers to the question: When do you visit the dentist?

The third group of questions was related to data on the presence of bad habits (Addendum 1). A quarter (22.5%) of the observed children nibbled the objects, 27.5% of them were fifteen year olds. Fifteen year olds had a higher percentage of developed pen nibbling bad habit in relation to the twelve year olds ($p < 0.05$), while boys more often without provocation grip and gnash their teeth.

Unilateral chewing usually indicates the existence of deep carious lesions on the painful side of the jaw. The number of children, who chew on only one side of the jaw, is not insignificant, 16.8% of them seem to do it unconsciously. Every tenth child consumes only softer food (10.1%). Among them, twelve year olds more than fifteen year olds prefer to eat refined, processed food, what indicates a statistically significant difference ($p < 0.05$). Fifteen year olds prefer fresh, unprocessed foods in their diet as opposed to twelve year olds. Boys are more frequent consumers of soft, sticky foods than girls ($p < 0.01$).

More than half of the children observed consumed conditionally cariogenic food (57.7%), while one third (36.0%) enjoyed eating cariogenic food. High statistical significance was observed by age ($p < 0.01$), while boys ate more chocolate and drink Coca-Cola ($p < 0.05$) (Figure 5).

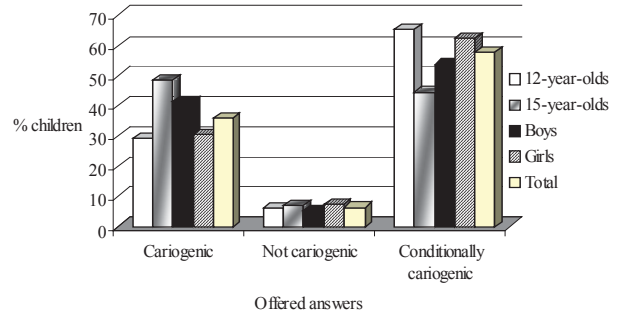

Fig. 5 – Distribution of answers* to the question: What do you prefer to consume?
***Answers grouped according to the influence of food type on tooth decay due to the statistical analysis.**

Discussion

Health education in the field of oral health is very important for acquiring satisfactory oral hygiene habits, but the relationship between knowledge and behavior is not coordinated. It seems that the improvement of the patient's health is more effected by the level of professional engagement, than the patient's knowledge [8].

Independently acquired improper habits of the patients belong to a group of local factors for the occurrence of gingivitis, since they contribute to the accumulation of dental plaque. Long-term retention of dental plaque on caries predilection sites can cause tooth decay over time.

Children often do not have a clear idea of why it is necessary to brush their teeth, and most do not think about the importance of prevention of oral diseases, which increases the risk of caries and gingivitis [9].

Applying the strategy of individual assessment of risk for caries, dentists can tackle the problem in relation to the target groups at risk for tooth decay, pointing to the risk factors, using special methods that include educational and motivational methods with the use of preventive prophylactic methods. All that should be included in everyday practice rather than just earlier approach of carious lesions treatment, which includes only detection of caries lesions and their treatment [10].

Oral health in twelve year olds is enhanced in the last few decades on the territory of our region. It is difficult to explain the reasons for this improvement because all preventive measures taken had been limited to local level. The authors of this study as reasons for oral health state improvement cite strict application of WHO guidelines for the diagnosis of caries lesions, and the use of WHO periodontal probe during examinations [11].

The participant of this study were informed about the factors that may affect oral cavity health, and about the way that it should be preserved. The children from the urban part of Banja Luka stated that they brush their teeth since the age of two, while their rural peers started with teeth brushing between 4 and 6 years of age [12].

Twelve year olds in China believe that the teeth should be brushed preventively in order to have healthy teeth in 76.5%, and for aesthetic reasons in 42%. In the same study, children reported the lack of time as the most common reason why they do not brush their teeth, and it is interesting that 3% of respondents consider teeth brushing useless because the good quality of teeth is hereditary [13]. Respondents from Kushinga (Malaysia) (70.3%) believe that dental plaque accumulated on the teeth cannot cause decay, while brushing teeth on regular basis can prevent gum bleeding (57.4%) [14]. Peers from Spain (79.8%) believe that with good oral hygiene, tooth decay can be avoided [15].

Teeth brushing, as a measure to preserve teeth and gingiva health, should be applied several times during the day (after getting up, after every meal, before bedtime), every day, while brushing the length should be adjusted to age for at least 5 minutes for older children. Similar data are presented by Pellizzer et al. [8] where 82% of girls brushed their teeth more often, in contrast with data from studies [16, 17] that found that girls and boys maintain their oral hygiene in about the same manner. Twelve year olds (28.1%) from this study brush their teeth longer, but most of the children (58.3%) are doing it for about 2 minutes. A total of 92.85% of the exam-

ined children from Sarajevo brush their teeth at least twice a day (Bosnian entity, Federation) and around 56% children from Banja Luka (Bosnian entity, Republic of Srpska), 18.5 % of children from rural part and 9.6% from urban parts of Banja Luka [10, 12, 18]. Their peers, 81.8 % of school children from Pančevo brush their teeth twice a day, 70% of Croats, 45% of Chinese brush their teeth, 50.2% of children from Saravak (Malaysia), 27.5% of girls from Saudi Arabia, about 69% of children from Northern Jordan, about 55.6 % of children from Portugal [8, 13, 14, 19–22] and in most of the cases brushing length is shorter than 2 minutes. The role of parents in proper development of oral-hygiene habits is significant. Children whose parents brushed their teeth twice a day also had similar habit [19].

Oral care tool set for these age groups should consist of tooth brushes, tooth paste with fluoride and dental floss. The level of awareness of the importance of oral hygiene and its maintenance, increases with the age of children and it is more pronounced among children living in urban areas [12, 19, 23].Twelve year olds living in Banja Luka maintain their oral hygiene with toothbrush and toothpaste [18]. A toothbrush as the only oral hygiene tool is used in 90.8% of twelve and fifteen year olds [24]. In the Grewala study, children mostly maintained their oral hygiene with tooth brush and dental floss [25]. Good oral hygiene of the parents and habits to control children while washing teeth are significant predictors of good oral health in children [19].

When it comes to awareness of fluoride, as one of the most powerful means in prophylactic dentistry, most children (93%) did not use fluoride tablets or any other fluoride means. Having in mind that there is no toothpaste that does not contain fluoride today, this is even more interesting information. First of all, we can say that it is due to the lack of children's knowledge gained by dentists, parents, teachers, means of mass communication, or perhaps lack of interest of children (parents). Data from this study are similar to data from the literature [13, 18, 19, 23], while 77% of Croatian adolescents were informed about the positive effect of fluoride on caries development [8]. Of all the fluoride preparations, toothpaste with fluoride is used by 4.3% of Sarajevo twelve year olds [10].

Drinking water in the municipalities where study was conducted has insufficient amount of fluoride (< 0.3 mg F/L). A recent study in the same municipalities indicates a high caries incidence of 5.64 in twelve year olds and 7.12% of fifteen year olds [26]. Latest guidelines on the use of fluoride do not recommend giving fluoride systematically to the whole population. This measure is recommended only to children with a high caries risk, what that is the case with the majority of children in this area.

The use of toothpaste with fluoride is a basic caries preventive measure that is recommended to everyone and should be promoted. In cases brushing is not implemented as recommended or caries risk is increased from any other reason, then additional sources of fluoride can be used [27].

The first dental appointment should be arranged in early childhood, just after the emergence of the first primary teeth (if there are no objective reasons for the earlier examinations), while all the teeth are healthy. However, 62% of respondents met with the dentist for the first time on regular examination before starting the school.

Bad experience from contact between the child and the dentist or medical workers', "fear of the white coats", will adversely affect the future behavior of the child to the dental treatment. Exaggerated and false stories about the "horrific and painful" interventions learned from other children or the parents can also create a false image in the head of little patient, that increases the fear of dental procedures. This kind of fear can be a significant limiting factor in maintaining oral health, which leads to the delay of dental visits and eventually to unsatisfactory oral health. Expression of dental fear increases in this way and creates a vicious circle [28].

Based on the results of this study the main reason for dental visits is a toothache (45%). A study conducted in Portugal shows that twelve year old girls from the city's private schools far more frequently visited the dentist [22]. The same study reported that 58.4% of six year olds and 13.3% of twelve year olds have never been at the dentists'. Literature review reveals that toothache is the most common reason for dental visits [14, 21], while the less frequent reasons are regular dental examinations or regular interventions [18, 24] or every six months visits [17]. In contrast to that, data obtained in studies at the area of Sarajevo, Banja Luka and Pančevo, show that children more often go for control check-ups, as well as for appointments with their dentist [10, 12, 19].

The existence of any long-term changes in soft or hard tissues can cause pain, which by itself prevents the regular, daily brushing, or the use of painful jaw while eating. Although 87% of respondents do not avoid brushing their teeth, the boys are doing it more often than girls. To the questions in the survey related to subjective symptoms such as edematous gums, bleeding from the gums and unpleasant breath, 95% of respondents answered negative. The percentage of 5 is not negligibly small, each 25th respondent feel discomfort in the area of one's mouth.

Slightly more than half of the respondents (50.2%) confirmed to breaththrough the mouth, that increases the risk for reduced secretion of saliva, increased percentage of gingivitis and tooth decay respectively. Over 80% of the respondents do not practice bad habits such as teeth griping and gnashing, unilateral chewing, the use of soft food or foreign objects nibbling. Fifteen year olds are more likely to practice a bad habit of biting foreign objects (27.5%).

Longtime experiences of numerous authors undoubtedly favor the claim that in patients with poor oral hygiene occurrence of caries is more frequent. In children with poor oral hygiene, eating sweets between meals is the most important risk factor in the development of caries. This was confirmed by a study conducted in Priština, where children with caries consumed more carbohydrates between meals, did not washed their teeth regularly and had higher values of plaque index, lower pH values of stimulated saliva compared to their peers with healthy teeth [29].

According to our research every tenth child consumes only softer foods. Among them, twelve year olds prefer to eat refined processed food. Boys are more frequent consumers of soft sticky foods. Studies conducted worldwide have

shown that children are informed about the effects of candy on dental health [8, 12, 19, 23, 24] but sweet meals, mostly chocolate, cakes, fizzy drinks are most frequently consumed, at least once a day [14, 15, 18, 24, 25, 30].

Conclusion

Children included in this study are relatively well-informed about the importance of health, oral health, use of appropriate tools for oral hygiene maintenance, brushing teeth length and its frequency. Surprising is the poor knowledge about fluoride application in caries prevention. Although children in this study acquired knowledge necessary for good oral health, based on the attitudes they have, their daily habits are inconsistent with their knowledge. The results showed that the relationship between knowledge and practice is contradictory. Therefore, knowledge transfer and control over implementation of that knowledge should be enhanced.

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Addendum 1

Layout of the survey questionnaire

Questionary for students (*General data*)

First and last name of the student: .....

School, grade, class: .....

(A) Knowledge of the children		
No.	Question	Answeres
1.	Why is it necessary to wash teeth?	To keep teeth healthy
		To keep teeth clean
		Because parens ask me to do that
		Other
2.	Do you have your own toothbrush?	Yes
		I do not have
		I have, together with my sister/brother
3.	Do you brush your teeth by yourself, and since when?	Yes (start a time when, ...)
		No, parents help me
4.	When do you wash your teeth and how many times a day?	In the morning or evening (once)
		After a meal (three times and more)
		When I leave house
5.	How many times teeth need to be washed a day?	Once
		Twice
		Three times
		Five times
6.	Do you wash your teeth and mouth regularly?	Regularly
		Occasionally
		I do not wash
7.	How long do you wash your teeth?	Around minute
		More than two minutes
		Around five minutes
8.	What means do you use for oral hygiene maintenance? *	Toothbrush
		Toothpaste with fluoride
		Toothpaste without fluoride
		Dental floss
		Toothpick
9.	Do you avoid tooth brushing?	Yes
		No
		Occasionally
10.	Did you use tablets or other preparation containing fluoride?	Yes
		No
11.	When did you visit the dentist for the first time?	When I was 3 years old
		Before starting the school
		At the age of 10
12.	When do you visit the dentist?	When I have a toothache (as necessary)
		Preventive controls
		When I have an appointment
(B) History data		
13.	Do your gums bleed when you wash your teeth?	Yes
		No
14.	Are you gums swollen or painful?	Yes
		No
15.	Do you feel bad smell (odor) from your mouth?	Yes
		No
16.	Are there some discomfort in the area of t he mouth and teeth?	Yes
		No
(C) Bad habits		
17.	Do you usually breathe on your mouth?	Yes
		No
18.	Do you scratch your teeth?	Yes
		No

19.	Do you often clench your teeth?	Yes
		No
20.	Do you nibble objects (pencil, nails, toothpick)?	Yes
		No
21.	Do you often chew food only on one side?	Yes
		No
22.	Do you use softer food in the diet?	Yes
		No
23.	What do you prefer to consume? *	Chocolate
		Fruit
		Vegetables
		Coca-Cola

*** Questions with answers grouped according to the accuracy or the impact of the type of food on caries, due to the statistical analysis of data.**

SHORT COMMUNICATION

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Antibiotic-loaded cement spacer for treatment of *Klebsiella* infected total hip and knee arthroplasty

Cementni spejser sa antibiotskim sadržajem za lečenje bolesnika sa totalnom artroplastikom kuka i kolena inficiranih bakterijom *Klebsiella*

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Abstract

Background/Aim. Infection following total hip arthroplasty (THA) or total knee arthoplasty (TKA) may have devastating consequences. Some bacterial strains are often encountered as agents of these infections, others occur less frequently but are sometimes burdened with more severe complications. *Klebsiella* spp. are uncommon causes of THA or TKA infection. The aim of this study was to identify an effective treatment algorithm for multidrug resistant *Klebsiella* spp. caused THA or TKA infections. **Methods.** During the 3-year period, from January 1 2009 to December 31 2011, we registered and treated 5 patients with THA or TKA multidrug resistant *Klebsiella* spp. caused infection. All the patients were primarily operated in other institutions, and were admitted in our clinic after the onset of infection symptoms. In three of the cases *Klebsiella* infection was complicated by additional infection (*Staphyloccocus aureus*, *Pseudomonas aeruginosa* and *Serratia marscescens*). In 3 of the cases we performed revision arthroplasty after double exchange of antibiotic-loaded articulating cement spacer, and in 2 of the cases the standard two-stage revision approach with one antibiotic cement spacer exchange was applied. **Results.** The mean length of follow-up after reimplantation surgery was 17.1 months (range 2–31 months). One patient died 2 months after the final reimplantation procedure. The initial *Klebsiella* infection was eradicated in all the patients. At the end follow-up after definitive reimplantation, the patients had no clinical, laboratory or microbiological parameters positive for active infection. **Conclusion.** According to our experience with multidrug-resistant *Klebsiella* TKA/THA infections, two-stage approach, in some cases with double articulating cement spacer exchange prior to definitive reimplantation, is the most effective treatment option.

Key words:
arthroplasty, replacement, hip; arthroplasty, replacement, knee; bacterial infections; klebsiella; orthopedic procedures; anti-bacterial agents; treatment outcome.

Apstrakt

Uvod/Cilj. Infekcije totalnih artroplastika kuka (THA) i totalnih artroplastika kolena (TKA) mogu imati teške posledice. Neke baterije su češći uzročnici, druge se javljaju ređe, ali su ponekad te infekcije opterećene težim tokom i komplikacijama. *Klebsiella* bakterije su retki uzročnici ovih infekcija. Cilj rada bio je identifikacija efikasnog algoritma za lečenje infekcija THA i TKA koje izaziva *Klebsiella*. **Metode.** U 3-godišnjem periodu, od 1. januara 2009. do 31. decembra 2011, registrovali smo i lečili pet bolesnika sa THA ili TKA infekcijom uzrokovanom multirezistentnim sojem bakterije *Klebsiella*. Svi bolesnici su primarno operisani u drugim ustanovama, a u našu kliniku su bili primljeni posle pojave znakova infekcije. Kod tri bolesnika infekcija bakterijom *Klebsiella* bila je komplikovana dodatnom infekcijom (*Staphyloccocus aureus, Pseudomonas aeruginosa* i *Serratia marscescens*). Kod tri bolesnika izvedena je reviziona artroplastika nakon dvostruke izmene antibiotskog cementnog spejsera, a kod dva bolesnika reviziona artroplastika sa jednom izmenom spejsera. **Rezultati.** Srednja vrednost perioda praćenja bila je 17,1 mesec (od 2 do 31). Jedan bolesnik preminuo je dva meseca nakon druge reimplantacione procedure. *Klebsiella* infekcije izlečene su kod svih bolesnika. Na kraju perioda praćenja nakon definitivne reimplantacija, bolesnici nisu imali kliničkih, laboratorijskih, niti mikrobioloških parametara pozitivnih na prisustvo aktivne infekcije. **Zaključak.** Nakon našeg iskustva sa TKA/THA infekcijama prouzrokovanim multirezistentnim sojevima bakterije *Klebsiella* ustanovili smo da je pristup sa dvostrukom izmenom artikularnih cementnih spejsera pre definitivne reimplantacije najefikasnija opcija lečenja.

Ključne reči:
artroplastika kuka; artroplastika kolena; infekcija, bakterijska; klebsiella; ortopedske procedure; antibiotici; lečenje, ishod.

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Introduction

Infection following total hip arthroplasty (THA) or total knee arthroplasty (TKA) may have devastating consequences for the patient. The incidence of infection associated with THA or TKA in many studies has been reported to range from less than 1% in general population to almost 4% in patient groups with comorbidities such as rheumatoid arthritis, that increase risk of infection [1–3].

Some bacterial strains are often encountered as agents of these infections, others occur less frequently but are sometimes burdened with more severe complications.

Bacteria belonging to the genus *Klebsiella* frequently cause human nosocomial infections. *Klebsiella* spp. are gram-negative, nonmotile, usually encapsulated rod-shaped bacteria, belonging to the family *Enterobacteriaceae* [4, 5]. These bacteria produce lysine decarboxylase but not ornithine decarboxylase and are generally positive in the Voges-Proskauer test. Members of the *Enterobacteriaceae* family are generally facultatively anaerobic, and range from 0.3 to 1.0 mm in width and 0.6 to 6.0 mm in length [5]. *Klebsiella* spp. often occur in mucoid colonies [4, 5]. The principal pathogenic reservoirs for transmission of *Klebsiella* are the gastrointestinal tract and the hands of hospital personnel. In particular, *Klebsiella pneumonia*, the medically most important *Klebsiella* species, accounts for a notable proportion of hospital-acquired urinary tract infections, pneumonia, septicemias, and soft tissue infections. It is estimated that *Klebsiella* spp. cause 8% of all nosocomial bacterial infections in the United States and in Europe [6]. Fortunately *Klebsiella* spp. are not common infective agents in TKA or THA infections, with a relatively small number of reports on the subject of *Klebsiella* periprosthetic infection in the literature [7, 8].

Two-stage reimplantation with antibiotic loaded cement spacers and 4–6 weeks of antibiotic treatment remains the most successful procedure for infection resolution. In most of the series success rates are up to 95 % [9, 10]. In cases of persistent multidrug-resistant (MDR) *Klebsiella* spp. infection there is a limited role for one-stage exchange and even two-stage reimplantation may not warrant eradication [11]. In some cases additional unconventional steps may be required for successful treatment.

The aim of this study was to identify an effective treatment algorithm for multidrug resistant *Klebsiella*-caused THA or TKA infections.

Methods

All investigations were conducted in conformity with ethical principles of research, and informed consent for participation in the study was obtained.

Since January 2009 to January 2012 we registered and treated 5 patients with THA or TKA multidrug-resistant *Klebsiella*-caused infection. All the patients were primarily operated in other institutions, and were admitted in our clinic after the onset of infection symptoms. The hospital records, operative notes, medications, laboratory reports, microbiological analysis data with antibiograms, and follow-up reports were reviewed. Collected data included patient age, gender, comorbidities, dates, initial arthroplasty procedure and subsequent reimplantation procedures, culture results with antibiograms, type of spacer, antibiotic combination mixed in the cement, *iv* and oral antibiotic therapy with the duration of antibiotic therapy.

There is the established protocol for the diagnosis and management of infected THA and TKA in our institution. It starts with preoperative clinical and laboratory evaluation. Aspiration is routinely performed if a patient was without antibiotics for at least 14 days prior to aspiration. If the results a positive for infection a patient is admitted and scheduled for surgery. All the patients in this series were initially planned for two-stage rearthroplasty. Preoperative and postoperative laboratory evaluation consisted of complete blood count, urine, biochemical analyzes, C-reactive protein, erythrocyte sedimentation rate, fibrinogen, and in some cases interleukin-6 was obtained. Intravenous (*iv*) antibiotic therapy was started after intraoperative cultures and tissue samples were obtained. In the first stage extraction of THA or TKA implants and debridement were performed followed by implantation of antibiotic loaded cement spacer (Figure 1).

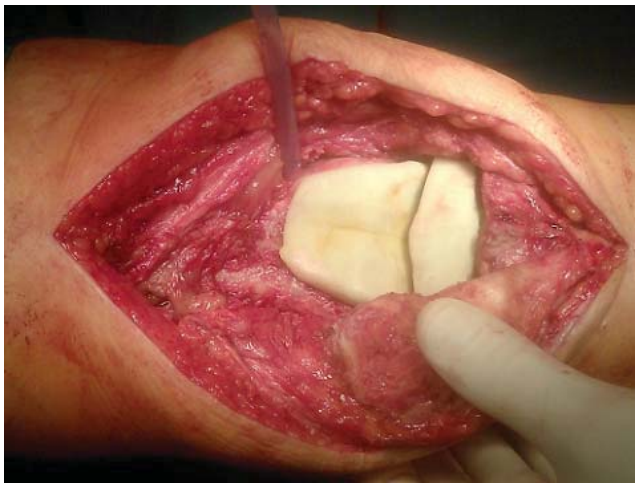

Fig. 1 – Articulating knee spacer.

In all the cases we used Refobacin® Revision (Biomet) bone cement containing a combination of two antibiotics: 1.0 g gentamicin and 1.0 g clindamycin *per* 40 g of bone cement. In one case where cultures, in addition to *Klebsiela* spp. were positive for *Staphyloccocus aureus* we intraoperatively added 2 g of vancomycin *per* 40 g of bone cement. In all the cases StageOne (Biomet) knee or hip spacer molds were used to make articulating spacer molds. After spacer placement no drains were used. Postoperatively, all the patients were regularly monitored by the infectious disease specialist, *iv* and afterwards oral antibiotic regimen was based upon pathogen sensitivity profile obtained from intraoperative cultures. Postoperatively, it consisted of combination of *iv* antibiotics in two cases, in other two cases *iv* meropenem 1 g every 8 h for two weeks, and in one case imipenem/cilastatin 0.5 g every 6 h for two weeks was applied. All patients underwent regular controls of renal, hepatic and hematologic

parameters during *iv* antibiotic therapy. *Iv* antibiotic therapy, was modified during treatment according to suggestions of infectious diseases specialist, but in all cases after spacer implantation duration of *iv* antibiotics lasted less than four weeks, and oral administration of antibiotics was continued afterwards. According to preoperative clinical, laboratory and intraoperative findings, second stage procedure with removal of articulating spaces had two possible outcomes, either final reimplantation of definitive prosthesis or three stage procedure with extraction of previous spacer, additional debridement and reimplantation of new knee or hip articulating spacer, and conditionally the 3-stage procedure with definitive reimplantation.

Klebsiella infections of THA and TKA in this study were found to be eradicated when at the end of a 12-month follow-up period after definitive reimplantation, the patients had no clinical, laboratory nor microbiological parameters positive for active infection.

Results

During a 3-year period, from January 1, 2009 to December 31, 2011, we registered and treated 5 patients with THA or TKA multidrug-resistant *Klebsiella*-caused infection. All the patients in the series had microbiologically confirmed *Klebsiella* prosthetic joint infection. During the treatment in 3 cases there were other pathogens isolated. All the patients were female, the mean age at the time of diagnosis of infection was 67.4 years (Table 1).

In 3 of the cases we performed 3-stage revision arthroplasty (double exchange of articulating cement spacer prior to reimplantation with definitive prosthesis), and in 2 cases 2-stage revision artheroplasty. The mean length of follow-up after the reimplantation surgery was 17.1 months (range 2 to 31 months); one patient died two months after the final reimplantation procedure. There were 3 infected THA and 2 TKA. The average time after primary arthroplasty and onset of infection symptoms was 28 days in the cases of THA and 39 days in the TKA cases. All the patients in the series had microbiologically confirmed *Klebsiella* prosthetic joint infection. In 2 of the cases of infected THA and in one case of infected TKA preoperative aspiration was positive for *Klebsiella*, and in other cases *Klebsiella* was isolated from intraoperatively obtained cultures and tissue samples.

During the treatment in 3 cases *Klebsiella* infection was additionally complicated by an infection with another bacteria. Two cases of *Klebsiella* infected total hips treated with double exchange of articulating spacer were complicated with additional pathogen, in one case during the first spacer exchange procedure *Pseudomonas aeruginosa* was obtained from intraoperative cultures, and in the other case *Staphyloccocus aureus* was found postoperatively in aspirations after first spacer implantation. One case of infected TKA was also treated with double spacer exchange, *Serratia marscescens* was isolated from knee aspirations after the first spacer exchange procedure. Adjustments in antibiotic therapy in these cases were made according to the resistance of *Klebsiella* and concomitant bacteria by an infectious diseases specialist.

Table 1

Patients characteristic and procedures

Patients characteristics (age, gender, Dg, Co)	Primary arthroplasty	Infecting bacteria	Secondary concomitant infecting organism	First spacer procedure and duration	Second spacer procedure type and duration	Second/third stage reimplantation
71 f; Dg: Osteoarthritis, Co: DM, urinary tract infection, hypertension	Cemented THA	*Klebsiella* spp.	*Pseudomonas aeruginosa* (isolated in cultures from first spacer implantation)	Articulating hip spacer for 12 weeks	Articulating hip spacer for 12 weeks	Eradicated, reimplantation-cemented THA
72 f, Dg: Rheumatoid arthritis; Co: DM, urinary tract infection	Cemented THA	*Klebsiella* spp.	*Staphylococcus aureus* (isolated from cultures obtained from aspirations after first spacer implantation)	Articulating hip spacer for 12 weeks	Articulating hip spacer (Refobacin cement with addition of vanco-mycin) for 12 months	Eradicated, reimplantation-hybrid THA
72 f; Dg: Femoral neck fracture; Co: hypertension	Cementless THA	*Klebsiella pneumoniae*		Articulating hip spacer for 8 weeks	None	Eradicated, reimplantation-cemented THA, patient died two months after final reimplantation
59 f; Dg: Osteoarthritis; Without Co	Bilateral simultaneous TKA (right knee got infected	*Klebsiella pneumoniae*	*Serratia marscescens* (isolated in cultures obtained during first spacer implantation)	Articulating knee spacer for 8 weeks	Articula-ting knee spacer (Refoba-cin cement) 3months	Eradicated reimplanted, cemented LCCK prosthesis
62 f; Dg; Rheumatoid arthritis; Co: DM, hypertension	Cemented TKA	*Klebsiella* spp.		Articulating knee spacer for 6 months	None	Eradicated, reimplanted cemented rotation hinge knee

Dg – primary diagnosis; Co – comorbidity; f – female; DM – diabetes mellitus; THA – total hip arthroplasty; TKA – total knee arthroplasty; LCCK – legacy constrained condylar knee.

One patient in the series with *Klebsiella* infected THA, 72 years old, was admitted to our hospital with evident signs of sepsis, severe anaemia and hepatorenal failure. This patient had cementless THA, at another institution, after femoral neck fracture, 5 weeks prior to admission in our hospital. Prior to sepsis development the patient was treated for two weeks with oral antibiotics at another outpatient clinic and was referred to our hospital when clinical and laboratory findings indicated sever deterioration. Besides obesity and high blood pressure before the primary arthroplasty the patient had no other comorbidities. This patient underwent 2-stage revision surgery, after first stage procedure and articulating spacer implantation there were significant positive improvements in the patient status. Hepatorenal failure persisted and required regular consults with nephrology and infectious diseases specialists. Eleven weeks after spacer implantation and subsidence of infection signs second stage procedure, cemented THA was perfomed. Initially early postoperatively patient was stable and recovering without complications. But one month after reimplantation cardiac and renal insufficiency developed, and the patient died 8 weeks after the reimplantation procedure. Considering reimplantation THA, at the time of death the patient was clinically, laboratory and microbiologically infection free.

One patient in the series, 59 years old, had bilateral simultaneous TKA, during primary arthroplasty procedure right knee was done first, and there was *Klebsiella pneumoniae* infection of the right TKA, the left knee was infection free. The onset of infection signs was 6 weeks after primary arthroplasty. This patient was treated with double spacer exchange. The first spacer was removed after 8 weeks, and the second one after 3 months. *Serratia marscescens* was isolated from knee aspirations after the first spacer exchange procedure. Antibiotic therapy was altered according to antibiograms by an infectious disease specialist.

In four of the cases end-stage procedures were performed as cemented total hip or knee arthroplasty, Refobacin® Revision (Biomet) bone cement was used in all the cases. In one case a hybrid total hip was definitive implant.

All the patients were allowed immediate full weight bearing. We did not note spacer fractures in any of the cases. In one case of *Klebsiella* THA infection after first stage exchange 4 weeks postoperatively hip spacer dislocation was noted (Figure 2).

The average time between removal of primary implants and definitive reimplantation was 6.8 months (range 2–15 months).

The initial *Klebsiella* infection was eradicated in all the patients, at the end follow-up after definitive reimplantation, patients had no clinical, laboratory or microbiological parameters positive for active infection. Clinically, at the last follow-up, except for the patient who died, in both cases of revision TKA knee, society functional score [12] improved from 30 to 90, and in cases of revision THA, Harris hip score [13] improved from 57.15 to 89.7.

We have noted the effects of articulating spacer cement abrasion phenomena (Figure 3.) and in each case thorough debridement and copious irrigation was perfomed during each step of the treatment.

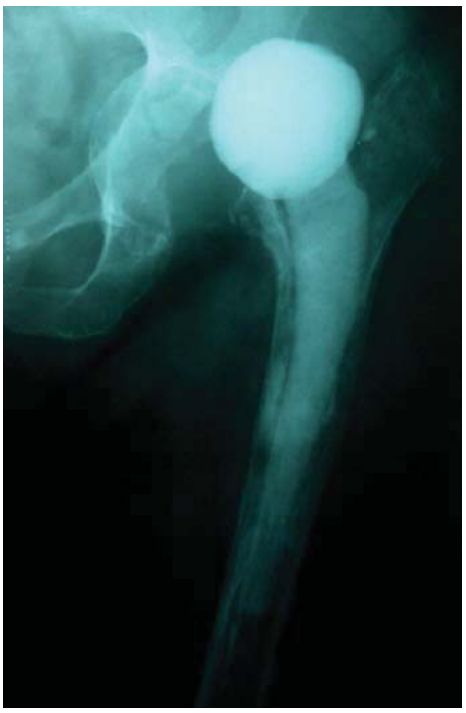

Fig. 2 – Dislocated hip spacer.

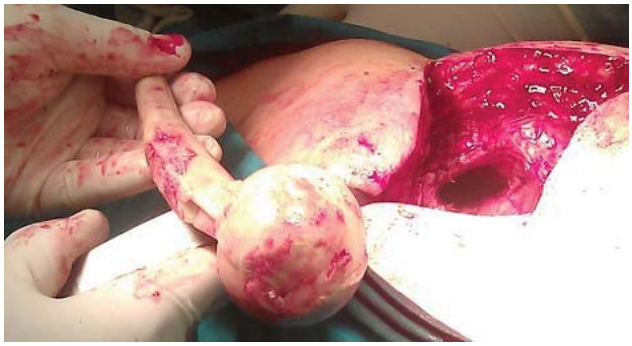

Fig. 3 – Removed articulating hip spacer – abrasions.

Discussion

The purpose of this study was to identify an algorithm of treatment of multidrug-resistant *Klebsiella*-caused THA/TKA infections. *Klebsiella* infections of TKA/THA are relatively rare [7–9], but can be very difficult to treat and sometimes can lead to sepsis, multiorgan failure and ultimately have fatal outcome.

There are few factors that additionally complicate treatment. *Klebsiella* infections of TKA/THAs can be complicated by an additional bacterial infection. Polymicrobial arthroplasty infections were seen in 6 of 10 knee arthroplasty patients; these infections involved a mixture of Gram-positive and Gram-negative species, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus species*, *Escherichia coli*, and *Enterobacter cloacae* [7, 14–20]. In our series in three cases *Klebsiella* arthroplasty infection was additionally complicated by an infection with another bacteria (*Staphyloccocus aureus, Pseudomonas aeruginosa* and *Serratia marscescens* were identified).

In addition to the multiresistant ability, *Klebsiella pneumoniae* has several adhesive factors, such as type 1 and type 3 pili, which help bacteria to adhere to abiotic or biological surfaces. The adherence of *Klebsiella pneumoniae* to cardiac valve prostheses, catheters of urinary tracts, intestinal cells, and bladder epithelial cells has been previously reported [21].

Two-stage treatment is currently the most common approach for management of an infected joint prosthesis, static antibiotic-impregnated cement spacers have traditionally been used, increasingly, however, mobile or articulating spacers are being utilized [22–24]. Although comparisons in the literature of static and articulating spacers have shown average eradication rates of approximately 90% and 92%, respectively [25], articulating spacers have some potential advantages, including more effective maintenance of the joint space and prevention of soft tissue contracture, facilitation of local antibiotic delivery, early mobilization, full or in some cases limited weight bearing, and possible reduction in bone loss.

In all cases we used antibiotic-loaded cement articulating hip and knee spacers. In our series of *Klebsiella* infected TKA/THA simplex two stage revision was not always sufficient option, and in some cases repetition of articulating antibiotic cement spacer prior to final reathroplasty was required for eradication of infection.

We used the term 3-stage reimplantation for the procedures of repetition of antibiotic cement spacer prior to reimplantation of definitive prosthesis. In the literature generally accepted understanding of 2-stage reimplantation concept, as it was firstly described by Insall et al. [26] and further promoted by other authors [14, 17, 27,] points to the procedures where the stage one is the operation with removal of infected implants and application of cement spacer, after a certain period of time followed by the stage two, spacer removal and definitive prosthesis implantation. Some authors consider as 2-stage reimplantation procedures even cases where two cement spacers exchanges occur before definitive rearthroplasty [7]. It could be that in situations where spacer elution time has subsided, but infection signs are still present and there are clear indications for one more spacer repetition, a more appropriate term is 3-stage reimplantation. This slight change in arthroplasty terminology could contribute to better recognition and follow-up of persistent periprosthetic joints infections caused by MDR bacteria treated by double cement spacer exchange.

Fink et al. [28] noted that articulating spacers used in 2-stage revision surgery of infected prostheses have the potential to abrade and subsequently induce third-body wear of the new prosthesis. Given the presence of abrasion debris, they recommend total synovectomy and extensive lavage during the second-stage reimplantation surgery to minimize the number of abraded particles and any retained bacteria.

Spacer fractures were reported [29, 30] but we had no fractured cemented spacer in the series.

Our study has some limitations. The major deficiencies are a small number of patients, and its retrospective design. Larger series and prospective research may be needed to provide adequate predictions for the appropriate treatment modality. The small number of patients, and heterogeneity of the series prevent us from making a definitive recommendation of which primary treatment is required for eradication of infection and restitution of function.

Conclusion

In our limited experience with multidrug-resistant *Klebsiella* total knee arthroplasty and total hip arthroplasty infections, we consider that 2-stage and 3-stage revisions (double articulating cement spacer exchange prior to definitive reimplantation) are the most effective treatment options.

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Endurance and resistance training in rehabilitation of patients with multiple sclerosis

Aerobni trening i trening sa progresivnim opterećenjem u rehabilitaciji obolelih od multiple skleroze

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multipla skleroza; rehabilitacija; vežbanje.

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system, affecting 2.5 million people worldwide [1]. It is the most frequent non-traumatic disabling neurological disorder among young adults [2]. Despite a number of immunomodulatory agents and various symptomatic therapies, there is still a significant level of disability and reduction of quality of life in this population. Therefore, rehabilitation treatment seems to be a very important therapeutical approach, having the potential to reduce symptoms and signs of disease, diminish level of disability and improve independency of MS patients.

Rehabilitation of MS patients is multidisciplinary and consists of kinesiotherapy treatment, occupational therapy, speech therapy, psychological treatment and social worker counseling. Although kinesiotherapy represents an important part of rehabilitation, the intensity, frequency, duration and type of exercises, have not been precisely defined so far [3]. Exercise programs are mostly individually tailored and directed towards the actual neurological symptoms and signs, such as impaired balance, coordination, walking capacity and muscle weakness [3]. It has been shown that MS patients show a reduced aerobic capacity [4, 5] and diminished isometric [6, 7] and isokinetic [8] muscle strength, not only due to insufficient activity and deconditioning [6] but also due to neural mechanisms including inability to activate the entire motor unit and a reduced firing rate of motor units [9].

Engaging MS patients into physically demanding activity has not been advised for a long time, because of fear that it could provoke worsening of symptoms and signs of disease [10]. Although more recent literature opposed that attitude [11], it still seems to be one of the biggest obstacles for massive implementation of endurance and resistance training in kinesiotherapy programs in MS treatment. It is very often seen, that even when these two trainings are encompassed in rehabilitation program, they are not performed in accordance with recommendation on intensity and duration that are necessary to achieve specific positive effects [12]. In such a way, many symptoms and consequences of the disease are left undertreated and rehabilitation potential is not fully utilized. Positive effects of endurance and resistance training are already well-studied in healthy population [13, 14], but recently published results showed that similar effects could be obtained in persons with MS if adequate training regimen were used [12]. Endurance and resistance training are well-known for its potential to improve muscle strength and condition. Resistance training improves muscle strength [15–18] and is a result of two mechanisms: hypertrophy of muscle fibers [19] and augmentation of efferent motor drive from spinal motor neurons to lower limbs [20]. Endurance training, on the other hand, improves aerobic capacity by influencing physiological adaptation of the cardiorespiratory system [21, 22]. Effects of endurance and resistance training on different symptoms and signs of MS, as well as on activity and quality of life, are much more studied in recent time and have promising results in many of these domains [17, 18, 21].

The aim of this paper was to review available scientific evidence regarding the effects and safety of endurance and resistance training in patients with MS and bring out current recommendations on their prescription. For this purpose we reviewed randomized control trials (RCT) and review articles that investigated the effects of endurance and resistance training in MS patients.

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Effects of endurance and resistance training in MS patients

Effects on functional capacity

This is the most studied domain of physical functioning, which represents the ability to perform activities of daily living [12]. Outcome measures used for this purpose vary across different studies, so it is very difficult to draw out solid conclusions regarding which kind of exercise better influence this function.

Resistance training showed favorable results in activities such as transfers from sitting to standing position or stair climbing [23, 24]. Positive effects on gait performance were seen only at high intensity resistance training regimens [23, 25] (Table 1).

Aerobic training influences greatly walking distance and speed, even when comparing to classical kinesiotherapy treatment [23, 26, 27] (Table 2). Its effects on other measures of functional capacity have been rarely investigated [12]. Analysis of different intensities of endurance training reveled that

Table 1

Effects of resistance training on muscle strength, functional capacity, fatigue, balance and quality of life in patients with multiple sclerosis (MS)

Study (references)	Sample size TG/CG	Study design	Disability (EDSS score)	MS course	Training			Training effect on study outcomes
					duration (weeks)	frequency (days/week)	intensity	
Dodd et al, 2011 [18]	36/35**	RCT	NR	RR	10	2	10RM/2ST	muscle strength: ↑↑ WHOQOL bref: ↑↑ MFIS: ↑↑
Learmonth et al, 2012 [32]	20/12	RCT	< 5.5	NR	12	2	40 min circuit training + balance exercises	TUG: ↑↑ ABCQ: ↑↑ 6MWT: Ø BBS : Ø
Dalgas et al, 2009 [23]	19/19	RCT	< 5.5	RR	12	2	15–8RM	muscle strength: ↑↑ FC: ↑↑
Dalgas et al, 2012 [36]	16/15	RCT	3.0–5.5	RR	12	2	15–8RM/3–4ST	FSS: ↓↓ MDI: ↑↑ PCS-SF36: ↑↑ MCS-SF: 36↑↑
Fimland et al, 2012 [20]	7/7**	RCT	< 5.0	NR	3	5	4RS/4RS	MVC: ↑↑ EMG activity: ↑↑
Cakt et al, 2012 [25]	15/15/15**	RCT	< 6.0	RR; SP	8	2	TG(1) bicycle ergometer + balance exercises TG(2) strength + balance CG: NI	TUG: ↑↑, DGI: ↑↑, FSS: ↑↑ FR: ↑↑, FES-↑↑ RPSF36 – ↑↑, BDI: ↓↓ 10MWS – Ø

****Different procedures; ↑↑ – significantly improved; ↓↓ – significantly decreased; Ø – a non-significant change; EDSS – Expended Disability Status Scale; RCT – randomized controlled trial; RR – relapsing remitting MS; NR – not reported; TG – training group; CG – control group; RM – repetition maximum; ST – set; WHOQOL bref – World Health Organization Quality of Life shorter version; MFIS – Modified Fatigue Impact Scale; FC – functional capacity; FSS – Fatigue Severity Scale; MDI – Major Depression Inventory; PCS – SF36 – Physical Component Score SF36; MCS-SF36 – Mental Component Score; NI – no intervention; FR – functional reach; TUG – Timed Up and Go test; DGI – Dynamic Gait Index; 10MWT – 10 minute walk test; RPSF36 – Role Physical SF36; BDI – Beck Depression Inventory; FES – Fall Efficacy Scale; ABCQ – Activity Balance Confidence Questionnaire.**

Table 2

Effects of endurance training on walking distance, fatigue, and quality of life in patients with multiple sclerosis (MS)

Study (references)	Sample size TG/CG	Study design	Disability (EDSS score)	MS course (patient number)	Training			Training effect on study outcomes
					duration (weeks)	frequency (days/week)	intensity	
Collet et al, 2011 [27]	20 /21/20*	RCT	NR	RR 22	12	2	TG(1) continuous TG(2) intermittent TG(3) combined	2MWT: ↑↑ muscle strength: ↑↑ TUG: Ø , FSS: Ø, SF36 : ↓
Dettmers et al, 2009 [26]	15/15**	RCT	< 3.0	PP3 SP4	3	3	NR	walking distance: ↑↑ MFIS: Ø, BDI : Ø, HAQUAMS: Ø
Oken et al, 2004 [34]	14/10/9**	RCT	< 6.0	NR	26	1	ergometer bicycle-until fatigue	MFIS: ↓↓ SF36: ↑↑
Rasova et al, 2006 [35]	36/24/19/16**	RCT	< 6.5	NR	8	2	ergometer bicycle 60% VO2 max, 30 min	MFIS: ↓↓ MSQOL: ↑↑ BDI: ↑↑
Sabapath et al, 2011 [29]	16/16**	RCT	NR	RR10 PR 3 SP 3	8	2	ET: NR RT: 6–8 RM, 2ST	6MWT: Ø MFIS : Ø, FSST : Ø BDI : Ø, FR : Ø SF36 : Ø, TUG : Ø

***Different training intensity; **different procedures; ↑↑ – significantly improved; ↓↓ – significantly decreased; Ø – a non-significant change; EDSS – Expended Disability Status Scale; RCT – randomized controlled trial; RR – relapsing remitting MS; NR – not reported; TG – training group; CG – control group; RM – repetition maximum; ST – set; WHOQOL bref – World Health Organization Quality of Life shorter version; MFIS – Modified Fatigue Impact Scale; FC – functional capacity; FSS – Fatigue Severity Scale; MDI – Major Depression Inventory; PCS – SF36 – Physical Component Score SF36; MCS-SF36 – Mental Component Score; NI – no intervention; FR – functional reach; TUG – Timed Up and Go test; DGI – Dynamic Gait Index; 10MWT – 10 minute walk test; RPSF36 – Role Physical SF36; BDI – Beck Depression Inventory; FES – Fall Efficacy Scale; ABCQ – Activity Balance Confidence Questionnaire.**

higher intensities had a better influence on gait parameters, but have been connected with a higher number of injuries and dropping outs [27].

Although the combination of endurance and resistance training seems to be logic therapeutic choice, its effect has not been extensively studied. Surprisingly, the existing literature does not reveal effects on muscle strength and aerobic capacity when two training modalities (aerobic or resistance) were combined of singular training approach [28]. Data on effects of kinesiotherapy programs which would combine specific exercises addressing actual neurological problem with resistance and endurance training are still missing. However, this approach, which can potentially influence all components of impairment, seems promising in an effort to improve functional capacity in MS patients.

Effects on balance

Several studies investigated the effects of resistance training on balance [29, 30] (Table 1). Regarding endurance training, it is important to include some kind of activity that constantly provokes balance maintenance. That means that treadmill as a therapeutic device can be utilized, whereas stationary bicycle or machines for elliptical training are of no use [31]. To our best knowledge, RCT evaluating effect of endurance training on balance in MS are still missing. On the other hand, there is a sufficient evidence showing that resistance training in combination with balance exercises is much more effective than balance exercises alone [25, 32].

Effects on fatigue

Fatigue represents one of the most frequent and most disabling symptoms. In previous decades it was believed that physical activity worsens fatigue and patients were suggested to rest in order to diminish this symptom [33] Nowadays, it is well-known that exercises are safe and that they can even lessen fatigue [12] However, the intensity, duration and type of exercises that could have the most beneficial influence on fatigue still need to be determined. The majority of studies published to date have examined the effects of endurance training on fatigue (Table 2), but only few of them showed some reduction of fatigue, although without significant difference compared to other interventions [34, 35]. They showed that training of moderate intensity and longer duration give better results than high intensity/low duration endurance training [33].

The influence of resistance training on fatigue was examined in a limited number of studies [36]. Although not generated on large number of patients, effects of this kind of exercise are promising [36] (Table 1). It is believed that possible effects of resistance training on fatigue could be explained through central motor activation which was found to be decreased in MS patients with fatigue [20, 37]. A connection between fatigue and suboptimal cortical output has also been found, which can explain worsening of symptoms and signs of disease after excessive training regime [38]. Possible physiological effects of exercises on fatigue also include neural growth factor stimulation and its effect on plasticity, as well as stimulation of anti-inflammatory cytokine production [39, 40].

Effects on cognitive status and disease progression

Cognitive dysfunction is seen in 43–65% of MS patients and is characterized by decreased mental processing speed and memory impairment [41]. As similar changes are seen in elderly persons, cognitive impairment in people with MS can be understood as an accelerated process of aging. Some studies on elderly population showed positive effects of endurance and combined training on cognitive function improvement [42]. Although researches on population of MS patients demonstrated a correlation between aerobic capacity and cognitive status, causal relationship has yet to be proven [43].

Another interesting finding is a possible relationship between exercises and disease progression. Preliminary reports on this subject show a correlation between exercising and functional and structural changes in the brain. Possible underlying mechanisms include increase in neurotrophic factors and cytokines [44], but further longitudinal studies are needed to better elucidate this relationship.

Effects on quality of life

Quality of life is a very important dimension of health, especially for people with chronic diseases. It has been shown that people with MS have poorer quality of life compared to persons suffering from many other chronic diseases [45, 46]. In recent past, many trials addressed this specific topic in order to influence improvements in this domain [47].

Evidence regarding effects of exercises presented in recently published meta analysis showed that endurance training for a period of 3 months had greater influence on quality of life compared to those performed over a longer time [48]. This is probably due to easier motivation of the patient over a shorter period of time. Evidences regarding the effects of other type of exercises on quality of life are still inconsistent [45] (Tables 1, 2).

Methodology: a continuing challenge

Current literature reveals a variety of effects of endurance and resistance training in patients with MS, varying from no effect to a variable degree of positive effects on different outcome measures (Tables 1, 2) Nevertheless, it is very difficult to draw out solid conclusion on exact training intensity and duration that would produce certain beneficial outcome. To show results of some intervention, it is advisable to present effect size (ES) of the intervention on outcome measures. Systematic review performed by Asano et al. [49] showed ES, a measure of an association between an intervention and an outcome, to vary in different studies from moderate to strong. However, due to a great diversity in implemented training protocols, different outcome measures, different number of studied patients with different disease phenotypes and different levels of disability, it is very difficult to draw out a definite recommendation on exact training intensity, duration and frequency that would produce certain beneficial outcome in MS patients [50]. On the other hand, many other rehabilitation approaches, including multidisci-

plinary rehabilitation, comprehensive exercise, occupational therapy, are also without sufficient evidences to guide optimal rehabilitation treatment but are, nevertheless, regularly advised in routine work [51, 52].

Current recommendation for exercise prescription in MS patients

Exercises are always individually tailored in accordance with patient needs and possibilities. Thus, the recommendations based on current literature research [11, 22, 53, 54], which are listed below, are only a framework for an individually created program.

Recommendation for resistance training

It is advised that resistance training should start with closed kinetic chain exercises, using training machines, if possible [12]. If this is not applicable, then the use of elastic bands or body weight as a load is recommended. Regarding patient's safety, it is better to include free weight exercises in later phases of training [12].

In the beginning, the target intensity should be 15 repetition maximum (RM), starting with 1 and increasing to 3 sets of exercises. After approximately 2 weeks, the load can be raised to 12 RM, with later possible increase in the number of sets to 4 [12, 53, 54].

It is recommended to have 2–3 trainings weekly in a 12-week period to allow changes that could provide longer lasting effects [55].

Recommendations on endurance training

Recommendations on endurance training in MS patients are in accordance with those for healthy adults [53], but progression should be carefully monitored and increased depending on patient capacity. Practical indicators used to determine the intensity of endurance training are the target heart rate, and the level of perceived exertion. The most accurate way to establish targeted heart rate is the heart rate reserve (HRR) method [53]. The level of perceived exertion (Borg scale) is a subjective measure which reflects patient's feeling of fatigue, and is frequently used in everyday clinical practice. Initial level of fitness is important in establishing minimum intensity of training. For sedentary people it is approximately 30% of HRR, while for patients with average to good fitness level, it is 45% of HRR [53]. For patients with MS it is shown that moderate intensities of training (40–60% HRR) are safe and effective [27]. Endurance training should start with a 5-minute warm-up, at low intensity training (20% HRR) to allow the cardiopulmonary system to adjust to the new demand. Then, a patient should easily progress towards the desired frequency, with work phase duration depending on the targeted frequency, or patient's perceived exertion. Following the work phase, the low intensity cool-down is performed for 5 minutes to prevent blood pooling, and to promote clearance of lactic acid [53]. In the beginning, progression should be obtained by increasing training volume (frequency and duration) and later by increasing intensity. Appropriate duration of training may range from 20 (or even less at the beginning) to 40 minutes. Suggested frequency is 2–3 times weekly over a 12-week period [12].

There are no consistent recommendations on combined training prescription. Insufficient scientific evidences regarding this type of training allows us only to claim its safety [56]. This kind of training is most often prescribed as a combination of two trainings, performed on alternate days [56]. There are some suggestions that exercising should start with resistance training first, in order to improve muscle strength. Once a certain muscle strength is gained, endurance training could be performed more efficiently [12].

Conclusion

Current scientific evidence demonstrates that endurance and resistance training of moderate intensity are safe for patients with MS in whom these two training modalities show positive effects on muscle strength, condition, functional capacity, balance, fatigue and quality of life. In order to optimize rehabilitation effects in MS patients it is necessary to implement these two training modalities in everyday clinical practice. However, future research is needed to determine the optimal exercise treatment regimens for endurance and resistance training in MS patients with different disease phenotypes and different levels of disability.

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CASE REPORTS

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A successful retrieval of stripped outer coating of J-tip diagnostic guidewire from the left popliteal artery during elective coronary angiography

Uspešno izvlačenje odljuštenog spoljašnjeg sloja dijagnostičke koronarne žice J-tipa iz leve poplitealne arterije tokom elektivne koronarne angiografije

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Abstract

Introduction. Entrapment and fracture of diagnostic or therapeutic devices within the coronary circulatory system are a rare, but increasing problem. **Case report.** A 70-year-old man was admitted in our clinic for coronary angiography before the planned aortic valve replacement. An arterial sheath was inserted in the right common femoral artery. After introducing a J-tip diagnostic coronary guidewire into the aorta and advancing a left Judkins diagnostic catheter over it, suddenly occured peeling off of the wire´s hydrophilic coating at the aortic arch level. Very soon, this outer coating of guidewire carried by the blood stream was entered into the left femoral artery, then into the left popliteal artery. This stripped part of guidewire was successfully caught and extracted out by using a goose-neck snare catheter. **Conclusion.** A sudden stripping of outer coating of a J-tip diagnostic hydrophilic coronary guidewire during coronary angiography is possible to manage quickly and successfully by the use of a simple cathether.

Key words:
coronary angiography; intraoperative complications; treatment outcome.

Apstrakt

Uvod. Blokiranje i fraktura dijagnostičkih ili terapijskih sredstava unutar koronarne cirkulacije je redak, ali rastući problem. **Prikaz bolesnika.** Muškarac, star 70 godina, primljen je u našu kliniku zbog koronarne angiografije pre planirane zamene aortnog zaliska. Arterijski uvodnik je najpre postavljen u desnu zajedničku femoralnu arteriju. Posle uvođenja J-tipa dijagnostičke koronarne žice vodiča u aortu i navlačenja levog Judkins dijagnostičkog katetera preko žice, iznenada je došlo do odljuštenja hidrofilnog sloja žice na nivou aortnog luka. Vrlo brzo ovaj spoljni omotač žice nošen krvnom strujom ušao je u levu femoralnu arteriju, a onda u levu poplitealnu arteriju. Odljušteni deo koronarne žice je uspešno uhvaćen i izvučen napolje korišćenjem katetera u obliku guščjeg vrata. **Zaključak.** Iznenadno odljuštenje spoljašnjeg sloja dijagnostičke hidrofilne koronarne žice sa J-vrhom tokom koronarne angiografije moguće je rešiti brzo i uspešno pomoću običnog katetera.

Ključne reči:
angiografija koronarnih arterija; intraoperativne komplikacije; lečenje, ishod.

Introduction

Entrapment and fracture of diagnostic or therapeutic devices within the coronary circulatory system are a rare, but increasing problem. These complications can occur in 0.2–0.8% of patients during interventional procedures in coronary arteries [1, 2]. Removing of the entrapped guidewire should be made as soon as possible after confirming such a case.

We report unusual case of stripping of the outer coating of a J-tip diagnostic hydrophilic coronary guidewire during elective coronary angiography.

Case report

A 70-year-old man was admitted to our clinic for coronary angiography before the planned aortic valve replacement. After a puncture of the right common femoral artery by the single-wall needle and placement a femoral sheath, a 0.035-inch J-tip Terumo diagnostic coronary guidewire was introduced and advanced into the aorta. A left Judkins diagnostic catheter advanced over the guidewire to the aortic root level. Suddenly, of the wire´s hydrophilic coating peeled off. We withdrew the catheter and the wire together, but the outer coating of the wire,

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forming a loop, remained in the descending aorta. The looped stripped part of guidewire was immediately tried to catch by the other J-tip guidewire, but unsuccessfully. Very soon, this outer coating of the guidewire carried by the blood stream was entered into the left femoral artery, then into the left popliteal artery. The other arterial sheath was inserted after antegrade arterial puncture into the left femoral artery. The stripped part of the outer coating of the guidewire travelled into the left popliteal artery to the knee level during that time (Figure 1).

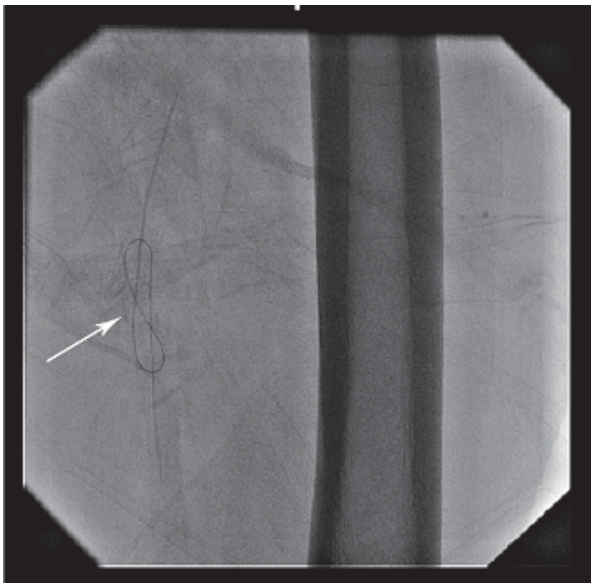

Fig. 1 – The stripped part of outer coating of diagnoistic J-tip guidewire in the left popliteal artery just at the knee level (arrow).

Then a goose neck snare catheter was introduced into the sheath and advanced it into the left femoral artery. After bypassing this stripped part of the guidewire we caught it and extracted it out (Figure 2).

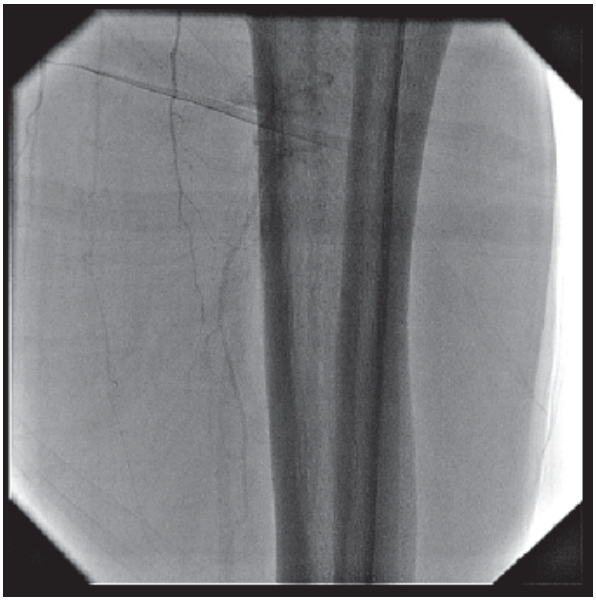

Fig. 2 – The left popliteal artery without outer coating of guidewire.

This outer coating was 18 cm in length (Figure 3).

The patient was without any symptoms, ECG and hemodynamic disturbances all the time.

Fig. 3 – Outer coating of guidewire 18 cm in length.

A parenteral antibiotic and 5,000 IU unfractionated heparin were added and the patient transferred to the coronary care unit for continuous monitoring. There were no symptoms or complications and he was discharged on the day 5.

A second admission and successful coronary angiography of this patient was made after 16 days. It was done by the right brachial approach without any problems. Coronary arteries were without any stenosis.

Discussion

There are 3 main components of guidewire structure: core, distal tip and outer covering. The inner part of the guidewire is referred to as the core. It extends through the shaft of the wire from the proximal to the distal part where it begins to taper. It is the stiffest part of the wire that gives the stability and steerability to the guidewire from its proximal end to the distal tip. The most popular core materials are stainless steel and nitinol. The coating is the outer covering on the core that keeps the overall diameter consistent and influences the wire performance. The type and length of coating may vary. Most often coating is applied to the distal 30 cm of the wire. Two types of coatings are used: hydrophilic coatings attract water and are applied over the entire working length of the wire, including tip coils. Hydrophobic coatings are silicone based coatings which repel water and are applied on the working length of the wire, with the exception of the distal tip [3].

In 63 (0.8%) of the 7,412 diagnostic coronary angiographies analyzed by some authors, periprocedural complications were observed. Periprocedural complications were divided into major and minor. Major complications included stroke, perforation of cardiac chamber, dissection or occlusion of coronary artery, dissection or hematoma of peripheral vessel, while the others were minor. The incidence of major and minor complications was 0.3% and 0.5%, respectively. Several risk factors associated with the occurrence of complications were noticed. Multivariate analysis showed that the size of catheters, combined left and right catheterization and the lack of experience of the physician were the most important [4].

It also describes the patient with the entrapped guidewire in the side branch after stent deployment in the main branch. The entrapped guidewire was surgically removed followed by coronary artery bypass grafting [5]. The retained guidewire was the reason for emergency coronary artery bypass in only one patient in the group of 118 patients [6] or in 15–20% of patients with failed percutaneous attempt to retrieve these remnants [7].

Hydrophilic coated guidewires are widely used in coronary interventions. Although they perform excellently in crossing tight and complex lesions, there is some risk of complications. The biggest potential complication is subintimal movement and dissection and perforation of coronary vessels. Another infrequent complication is fragmentation and entrapment of the guidewires. It can lead to an acute ischemic event due to thromboembolic occlusion. Retained guidewire fragments in the coronary tree cause complications such as emboli, thrombosis, dissection and rupture. The best management of an entrapped guidewire is still unclear. Surgical management, percutaneous extraction of a guidewire, stent implantation over the guidewire remnants and conservative follow-up can be chosen as a treatment [8].

The single-wall needle is preferred by most interventionists; its beveled leading point, advanced toward the vessel, finding the lumen on the way in. It represents the most important potential disadvantage of this needle type, as the long bevel can be partially placed within the vessel wall itself, while still obtaining adequate pulsatile blood return. If not recognized, this can lead to subintimal dissection of the punctured vessel. The second potential risk relates to the possible peeling off of the wire´s plastic or hydrophilic coating by the sharp needle point [9].

There are several methods for removal a broken diagnostic wire, including hook-tip catheters, snare loops, tip deflecting wires, balloon catheters, or grasping forceps. The Dormia basket is also used for successful retrieval of intravascular foreign body objects in 96% of cases [10]. Using these techniques is successful and safe, providing avoiding traumatic surgical removing these foreign objects.

Conclusion

A sudden stripping of the outer coating of a J-tip diagnostic hydrophilic coronary guidewire during angiography is possible to manage quickly and successfully by the use of a simple catheter. Our experienced physician used the single-wall needle and many times utilized and resterilized guidewire, what allowed stripping of the outer coating of the guidewire. The single-wall needle was already described as a possible cause of peeling off of the wire´s hydrophilic coating. In our opinion, these are the most important issues in this complication. Fortunately, this case was terminated without any consequences.

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CASE REPORT

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Uretrorenoscopy laser lithotripsy treatment of stones impacted in the left ureter 10 years after right kidney autotransplantation

Ureterorenoskopska laserska litotripsija u lečenju kamenova impaktiranih u levom ureteru deset godina nakon autotransplantacije desnog bubrega

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Abstract

Introduction. Urinary tract calculosis is a very common condition in general population. It appears in 5–10% of population, and can be managed conservatively or by minimally invasive, endoscopic and surgical procedures or extracorporeal shock way lithotripsy. Lesions of the ureter can be resolved by JJ stent insertion, end-to-end anastomosis, ureterocystoneostomy, percutaneous nephrostomy, nephrectomy, intestinal graft interposition or kidney autotransplantation. **Case report.** We presented surgical treatment and outcome in a female patient, with a large defect of the right ureter due to impacted stone treatment, following a successful autotransplantation of the right kidney. Ten years later a stone impacted in the left ureter was successfully treated by ureterorenoscopy and laser lithotripsy. Asynchronously combined kidney aoutotransplantation and ureterorenoscopic lithotripsy preserved kidney function. **Conclusion.** Bilateral organs preservation should be considered even in the absence of malignancy, especially in younger population.

Key words:
ureterolithiasis; lithotripsy, laser; transplantation, autologous; kidney.

Apstrakt

Uvod. Kalkuloza urinarnog trakta sreće se veoma često u opštoj populaciji. Javlja se kod 5–10% populacije i može se zbrinjavati konzervativnim pristupom, minimalno invazivnim endoskopskim ili hirurškim procedurama ili razbijanjem kamena udarnim talasima van tela. Oštećenja uretera mogu biti rešavana plasiranjem ureteralnih sondi, terminoterminalnom anastomozom uretera, ureterocistoneostomijom, perkutanom nefrostomijom, interpozicijom grafta creva ili autotransplantacijom bubrega. **Prikaz bolesnika.** Prikazali smo tok i ishod lečenja bolesnice sa velikim defektom desnog uretera nastalog kao posledica lečenja zaglavljenog kamena, sa naknadnom uspešnom autotransplantacijom desnog bubrega. Posle deset godina, kamen se zaglavio u levom ureteru i uspešno je razbijen ureterorenoskopskom laserskom litotripsijom. Asinhrona autotransplantacija bubrega i ureterorenoskopska litotripsija su rezultovali očuvanjem bubrežne funkcije bolesnice. **Zaključak.** Očuvanje parnih organa je opcija lečenja i u odsustvu malignih oboljenja, naročito kod mlađih bolesnika.

Ključne reči:
ureterolitijaza; litotripsija, laser; transplantacija, autologna; bubreg.

Introduction

Urinary tract calculosis appears in 5–10% of general population and can be managed conservatively, by Extracorporal shock way lithotripsy (ESWL), ureterorenoscopic lithotripsy, open or laparoscopic surgery, as well as by percutaneous nephrolithotripsy [1]. Lesions of the ureter account for 1% of all urinary tract lesions. Most often they are iatrogenic, caused by gynaecological interventions, abdominal surgery and urological treatment – open surgery or ureterorenoscopy [2]. Large defects of the ureter may be treated surgically, performing transureteroureterostomy, the psoas hitch method, Boari flap, nephrectomy, renal autotransplantation or with intestinal tract graft interpositions [2].

Case report

A 47-year-old female patient experienced renal colic type pain on the right side accompanied by a high-grade fever in June 1999. Diagnostic procedures and treatment were initiated in the United States and showed the presence of a suppurative pyelonephritis accompanied by II degree hydronephrosis and right-sided ureterolithiasis. Antibiotics were administrated and a JJ stent inserted into the right ureter.

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One month later the patient was hospitalised at our institution because of urosepsis and pyelonephritis of the right kidney. Sonography of the kidney showed II/III degrees ureterohydronephrosis on the right side. The findings of the left kidney were without any peculiarities. A kidney, ureter and bladder (KUB) radiography revealed that the superior end of the JJ stent lied outside the ureter. Antibiotic treatment was initiated and the JJ stent removed. A percutaneous nephrostomy catheter was inserted into the renal pelvis of the right kidney. In the vicinity of the lateral process of the L4, a knee bend in the ureter with a funnel-like taper was observed that was impassable for the contrast media (Figure 1). The

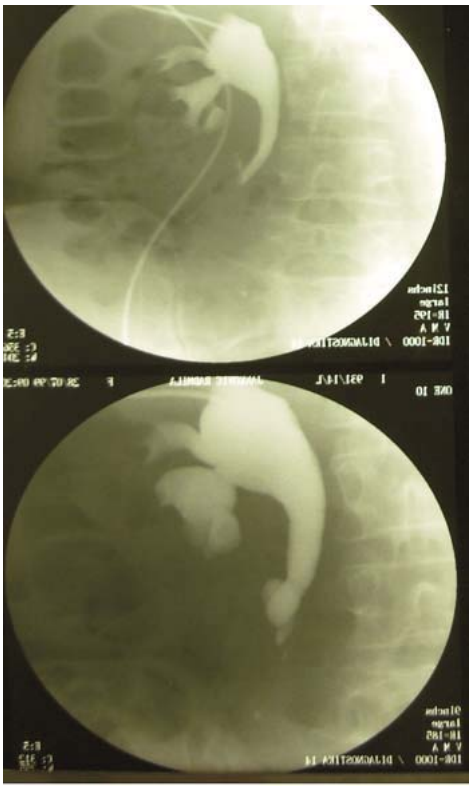

Fig. 1 – Anterograde urography, right side, showed hydronephrosis on the right, knee bend of the ureter in the vicinity of the lateral process of L3, the distal segment of ureter did not show.

patient was operated on 5 September 1999 when periuretral fibrosis was found with complete occlusion of the lumen of the right ureter, running 15 cm from the ureteropelvic junction to the vicinity of the iliac blood vessels. An autotransplantation of the right kidney was performed into the right iliac fossa. A control sonogram was performed on the day 4 following the operation and it showed no hydronephrosis or calculosis of the autotransplanted kidney. On the postoperative day 16 the patient was discharged home, afebrile, with normal laboratory test results and proper depuration.

In December 2009, the patient reported to the urologist for renal colic type pain on the left side. Renal sonography revealed III degree hydronephrosis of the left kidney. A stone measuring 12 × 6 mm was observed below the left ureteropelvic junction. Intravenous urography showed the right autotransplanted kidney in the pelvis minor, excreting in a timely fashion and proper concentrations, without hydronephrosis. Hydronephrosis was seen on the left kidney and the left ureter did not appear (Figure 2). The patient un-

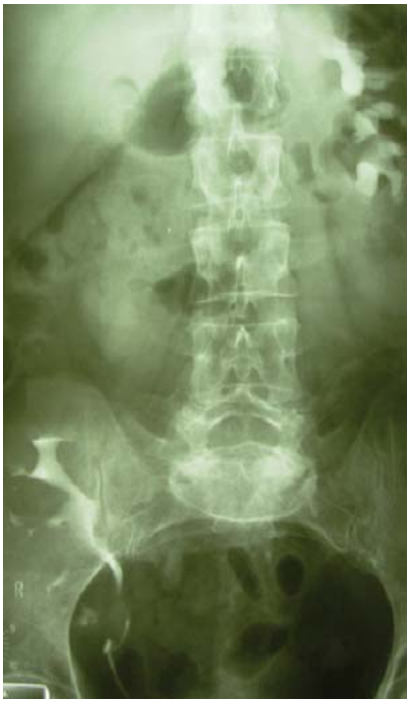

Fig. 2 – Intravenous urography performed in December 2009 showed hydronephrosis of the left contralateral kidney. The right autotransplanted kidney in the pelvis minor without urinary stasis. Both kidneys excreted contrast media. The left ureter did not show.

dervented ureterorenoscopy with laser lithotripsy of the stone in the left ureter with insertion of a JJ stent into the left ureter. The JJ stent was removed three weeks postoperatively. Three months after the surgery the patient had no longer any of the complaints and intravenous urography findings were without any peculiarities (Figure 3).

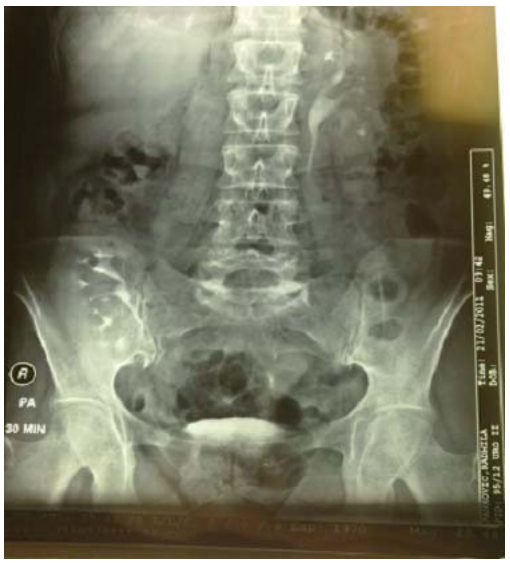

Fig. 3 – Intravenous urography performed in February 2011 showed no peculiarities on either kidney, pyelocaliceal system or ureter.

Discussion

Large defects of the ureter may be treated surgically by interposing the small intestine [2, 3]. This surgical method is accompanied by a number of complications: frequent urinary infections, improper renal function, electrolyte disorders and intestinal obstruction [4]. In cases when anatomical and technical considerations do not allow the use of an intestinal segment or other method for replacing the missing part of the ureter, renal autotransplantation may be the method of choice [2–5]. This is of a particular significance among patients with the anatomically or functionally solitary kidney. In the presented patient ureterocystoneostomy was not possible due to the size of the defect of the ureter, nor was possible to obtain a long enough graft of the bladder. Studies have shown excellent results and 93% preservation of the function of the autotransplanted kidney [4–7]. Thrombosis of renal blood vessels of autotransplanted kidneys is the most serious complication that appears in 0.5–4% of cases [8]. Ten years after autotransplantation, patient had laser ureterorenoscopic lithotripsy on the other kidney. Until the 1980s ureterolithotomy was the only treatment for stones in the ureter. With the development of ESWL and ureterorenoscopy, the indications for ureterolithotomy were brought down to minimum, with only 1–5% of patients requiring open surgery [9]. Complete disintegration and elimination of calculi is achieved among 93.1–97.7% of patients by URS laser lithotripsy and/or ESWL, methods that are painless, effective and have low rates of serious complications [10].

Kidney function deterioration was prevented in the presented patient by a proper combination of conservative treatment, minimaly invasive procedure, open surgery and endourological intervention.

Conclusion

Kidney preservation is indicated whenever it is possible, even in the absence of malignancy.

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CASE REPORT

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Unusual metastasis of esophageal cancer

Neobična metastaza karcinoma jednjaka

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Abstract

Introduction. Carcinoma of the esophagus is in the eighth place by the frequency of malignant diseases and the sixth cause of death from cancer worldwide. It usually metastasizes to regional lymph nodes, liver, lungs, central nervous system, and bones, but metastases can appear to unusual locations such as facial skin and lips. **Case report.** We presented a 56-year-old man who reported to his physician because of upper lip swelling. A physical checkup of the patients also showed a lesion on the skin of the left temporal region and both lesions were biopsied. Based on the results of histopathological and immunohistochemical analyses of the samples a diagnosis of metastatic adenocarcinoma to the skin was established. Additional diagnostic procedures, including esophagogastroduodenoscopy, detected the infiltration into the distal part of esophagus, which was histopathologically confirmed as adenocarcinoma of esophagus. The results of positron emission tomography/computed tomography (PET/CT) examination showed the invasion of the disease. Because of the disease expansion, a multidisciplinary oncology team suggested chemo- and radiotherapy treatment. The patient has received 4 cycles of platinum-based chemotherapy so far. **Conclusion.** The physicians should always consider unusual skin lesions as the first sign of cancer spreading.

Key words:
esophageal neoplasms; neoplasm metastasis; lip; diagnosis; adenocarcinoma; histological techniques; immunohistochemistry.

Apstrakt

Uvod. Karcinom jednjaka je osmi karcinom po učestalosti i šesti uzrok smrti od karcinoma u svetu. Najčešće metastazira u regionalne limfne čvorove, jetru, pluća, centralni nervni sistem, kosti, ali se sreću metastaze i na neuobičajenim mestima kao što su koža lica i usne. **Prikaz bolesnika.** Prikazali smo bolesnika starog 56 godina koji se javio lekaru zbog otekline u predelu gornje usne. Pregledom je viđena i promena u predelu kože slepoočnog predela glave sa leve strane te su u istom aktu ekstirpirane obe promene. Patohistološkim pregledom, uključujući imunohistohemijsku analizu, postavljena je dijagnoza metastatskog adenokarcinoma u koži. Dopunskom dijagnostikom, uključujući ezofagogastroduodenoskopiju viđena je infiltracija distalnog dela jednjaka, čiji je patohistološki nalaz potvrdio dijagnozu adenokarcinoma jednjaka. Pozitronska emisiona tomografija-kompjuterizovana tomografija (PET/CT) je ukazala na proširenost bolesti. S obzirom na proširenost bolesti multidisciplinarni onkološki tim je predložio hemioterapiju i radioterapiju. Bolesnik je do sada primio četiri serije hemioterapije po protokolu sa platinom. **Zaključak.** Lekari moraju imati na umu da neuobičajene promene na koži mogu biti prvi znak proširenosti karcinoma.

Ključne reči:
jednjak, neoplazme; neoplazme, metastaze; usna; dijagnoza; adenokarcinom; histološke tehnike; imunohistohemija.

Introduction

Carcinoma of the esophagus is sixth cause of death from cancer worldwide (fifth cause from cancer among men and eighth among women). China, countries of Central Asia, and certain parts of America are the regions with the highest incidence. In Europe, carcinoma of the esophagus is a rare malignant disease [1]. In Serbia about 460 newly diagnosed patients are registered annually [2]. The highest incidence is in the region of Vojvodina and it ranges 7 new cases *per* 100,000 yearly. Mostly it affects older population in the sixth decade of life. Adenocarcinoma of the esophagus most frequently metastasizes to regional lymph nodes, liver, lungs, central nervous system, bones and rarely to facial skin and scalp.

Case report

A 56-year-male patient visited the physician because of the skin lesion on his upper lip and skin of the left temporal region. Both lesions were biopsied and the samples were

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histopathologically analyzed. Based on routine hematoxylin and eosin staining (Figure 1) the diagnosis of metastatic adenocarcinoma was established. Because of the immunoprofile of tumor cells [cytokeratin (CK) 7 positive (Figure 2), epithelial membrane antigen (EMA) positive (Figure 3 and 4), carcinoembryonic antigen epithelial membrane antigen (CEA) positive, CK20, and thyroid transcription factor-1 (TTF-1) negative] additional immunohistochemical analysis of tumor tissue was done. The results presented as adenocarcinoma metastasis to skin.

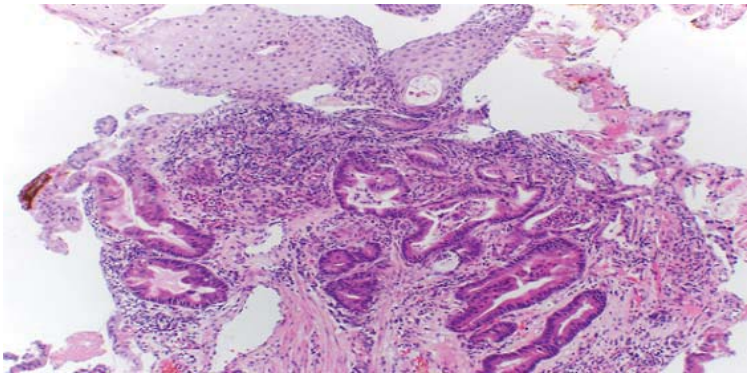

Fig. 1 – Mucosa and submucosa of the esophagus infiltrated by tumor tissue (hematoxylin and eosin staining, × 10).

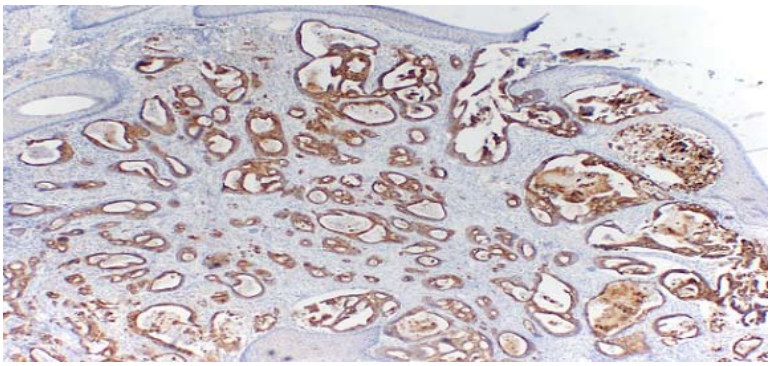

Fig. 2 – Tumor tissue infiltration into the skin of the upper lip (Cytokeratin7+, × 4).

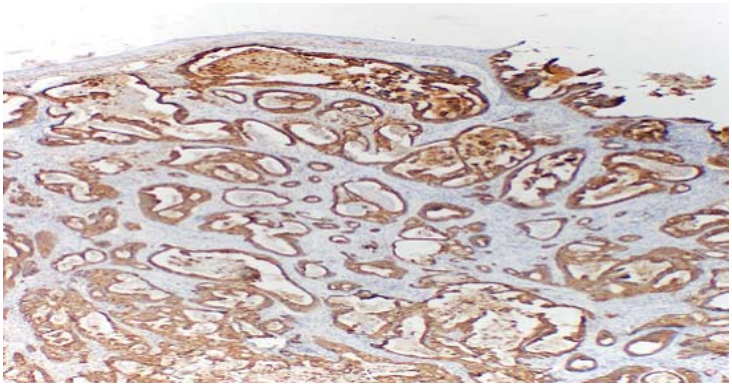

Fig. 3 –Tumor tissue infiltration into the skin of the upper lip (Epithelial membrane antigen +, × 4).

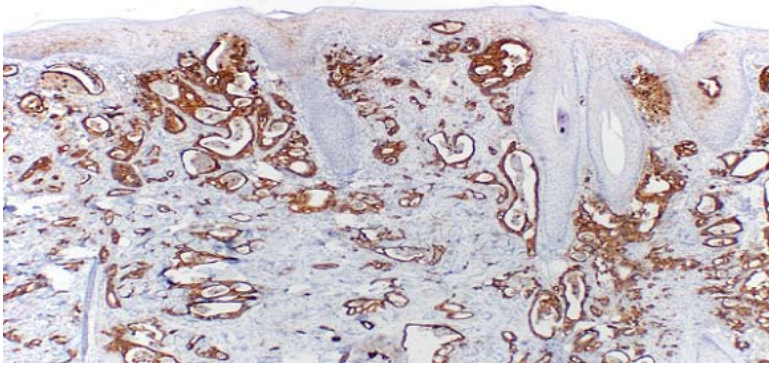

Fig. 4 – Tumor tissue infiltration into the skin of the left temporal region (Epithelial membrane antigen +, × 4).

Differential diagnosis procedure referred to primary adenocarcinoma of gastrointestinal tract. The patient has been suffering from rheumatoid arthritis for 10 years. He consumes alcohol moderately and quitted smoking 27 years ago. He smoked about 15 years on the average 30 cigarettes *per* day. As he complained of swallowing difficulties esophagogastroduodenoscopy was performed. The examination results showed an infiltrative stenosis of esophagus at 38 cm of its length, which was impassable for the tip of the instrument. The lesion was biopsied and the sample was histopathologically analyzed. The result showed a well-differentiated adenocarcinoma. The findings of computed tomography (CT) examination of the chest and abdomen indicated the presence of single lymph nodes in mesentery up to 6 mm in size and one small lymph node conglomerate of 14 × 8 mm. Bilateral diffuse nodular lesions found in lung parenchyma were most probably secondary deposits. In addition, CT findings evidenced lesion infiltration into the distal part of the esophagus and cardia. The patient was referred to positron emission tomography (PET)-CT examination by the surgeon to evaluate the expansion of the primary disease. The findings of the examination showed metabolic activity of lymph nodes in the axilla, bronchopulmonary segment right, preaortic space, and bilaterally in mesentery. The active focuses were also detected in the liver right lobe, skeleton (left arm, right shoulder blade, rib 9 right, cervical vertebra 7, lumbar vertebrae 1, 2, and 4, pelvic bones bilaterally, and proximal ends of both femur. Thyroid gland was polynodally changed and enlarged. A multidisciplinary oncology team indicated the treatment with chemotherapy (adriamycin 40 mg/m^2 at the day 1; 5-fluouracil 300 mg/m^2 at the day 1–5; cisplatin 20 mg/m^2 at the day 1–5, every four weeks) and radiotherapy. So far, the patient was administered 4 cycles of chemotherapy, not associated with any significant side effects. The patient reported smaller swallowing problems. Radiotherapy has not been initiated yet.

Discussion

The incidence of esophageal adenocarcinoma is significantly increasing [3, 4]. Most patients present at the advanced stage where therapeutic measures with a curative intent are not feasible [5]. Carcinoma of the esophagus characterized with biologically aggressive course, local infiltration, involvement of adjacent lymph nodes, and distant metastases by means of hematogenous routes [6]. Most frequently it metastasizes to lymph nodes (45%), liver (35%), lungs (20%), skeleton (10%), peritoneum (2%), and brain (1.5%) [7]. In total of 4,000 cases of metastatic esophageal carcinoma skin metastases were found in about 10% of patients [7]. Squamous cell carcinoma and adenocarcinoma of the esophagus metastasize to the skin with and equal frequency in less than 1% of cases. Similar to our case, Nisi et al. [8] report a case of esophageal carcinoma with upper lip metastases. The patient was confirmed to be affected with adenocarcinoma of the gastroesophageal junction with secondary deposits. Iwanski et al. [9] describe a patient with disseminated and extensive skin lesions. Histopathological analyses of these lesions show the presence of esophageal carcinoma. Maheshwari et al. [10] report a case of a female patient with skin lesions and difficulties in swallowing. Adequate diagnostic procedures and histopathological analysis of the lesions confirm skin metastases. Herbella et al. [7] describe a case of a patient with dysphagia and painless but rapidly advancing ulcerations on nose and neck, which all were histopathologically verified as metastases from squamous cell carcinoma of the esophagus. There is also a report on a patient presented with skin lesions two years after the operation of adenocarcinoma of the gastroesophageal junction; the lesion was histoptahologically verified as adenocarcinoma [7]. Metastasizing of esophageal adenocarcinoma to the upper lip is rare and with poor prognosis because of the high probability of secondary localization of the disease [8]. In total a 4-year survival of patients affected with carcinoma of the esophagus, regardless the stage of the disease, is lower than 10%, and 21% after the surgical treatment [11].

Conclusion

Physicians should always consider unusual lesion of the skin, which may be the first sign of disease expansion.

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The first pharmacy in Vranje with the educated pharmacist and its development

Prva apoteka u Vranju sa diplomiranim farmaceutom i njen dalji razvoj

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Key words:
pharmacy; history, 19th century; history, 20th century; legislation, pharmacy; community pharmacy services; pharmacists; serbia.

Ključne reči:
farmacija; istorija, XIX vek; istorija, XX vek; zakonodavstvo, farmacija; farmaceutske službe, komunalne; farmaceuti; srbija.

Introduction

Health conditions in Vranje during the Ottoman rule were similar to the situation in the rest of Serbia at that time with poor organized health services. People asked for help for the treatment of "hakims", barbers, doctors and self-educated folk healers. In the stores that were out of control, in addition to other goods, many medicines, raw materials for making medicines and poisons could be procured from Greece and Turkey. In the 20th century people could still remember various balms, splash, teas, syrups and "madzun" that could be bought in the shops of that time. At the beginning of the 19th century in Vranje, as well as in Serbia, the only help for the sick was provided by Greek self-taught healers, "kaloijatri". There was a self-taught folk healer Mika Stošić who became known in Vranje as "hakim"Mika. "Hakim" Mika died in Constantinople in 1854, where he had gone in order to solve the national status for the Municipality of Vranje [1]. He conveyed his knowledge to his sons Zafir and Dimitrije. Dimitrije was successfully practicing medicine for ten years after his father's death, until a prominent Turk child who was treated by Dimitrije died. Fearing that he would be blamed for the child's death, "hakim Dimitrać" as he was called, committed suicide [1]. A Turkish medical assistant Naum Marković, who graduated from Medical School in Constantinople, came to Vranje in 1862 [1]. Being a medical assistant (at that time a Turkish title "felcer" was used for this educational degree), he had the right to examine and treat patients and to prepare and sell medicines. In his two-floor house he opened an outpatient department and a pharmacy store where he prepared various preparations in the form of decocts and infusions and many others. Felcer Naum regularly went to Thessaloniki and Constantinople in order to supply his shop with the necessary materials and drugs of herbal, animal and mineral origin.

The aim of this paper was to present the historical development of the first community pharmacy in Vranje with the focus on its personnel, legal conditions and pharmacy regulations from the second half of the 19th and the first half of the 20th century.

The method of historical analysis was employed. Manual documentary analysis of original sources and desk research analysis from the secondary sources were conducted.

Health conditions before the foundation of the first pharmacy in Vranje

Development and significance of pharmacy in the health service in Vranje after the liberation from the Turks should not be considered separately from the economic, social and political conditions in Vranje in the late 19th and early 20th century. In the last year of Ottoman Empire in January 1877 in Vranje came a Greek named Dr. Atanasije Kujas, Athene's pupil with less experience gained in Constantinople and Jedrene [1, 2]. He was the only graduate doctor of medicine in that region. After the liberation from Ottoman Empire, he remained there to work as a physician until his retirement in 1902 [1]. After a year he died in Vranje. The people of Vranje acknowledged him for his devoted contribution in 1897 when was elected to be a mayor of the Municipality of Vranje [1]. Only for a while between May, 1 and Oct, 27 1882, he didn't work in Pčinje Canton because was

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appointed to be a physician for the neighbouring Poljanički Canton and he worked in Vladičin Han [2].

After the liberation from the Turks on January, 31 1878, the economic and cultural development of Vranje and its suburbs was slow. Specific topographic position of Vranje district as a crossroad of people and culture has always attracted numerous invaders. The frequent territorial division of Vranje district left serious political and economic consequences on the already impoverished city of Vranje. According to the decision of the Congress of Berlin a territorially unified space of Vranje Municipality was divided into two parts: Vranje belonged to Serbia, while a large part of Vranje district remained behind the boundaries which formed the Preševo "kaza" until 1912. After the liberation of Vranje from the Turks handicrafts, commerce, education and culture were experiencing major progress. The vicinity of Macedonia, Greece and Bulgaria contributed to overall economic and cultural progress. Trade relations into Vranje were started on the initiative of reputable retailers and Vranje became a city of craftsmen, merchants, and innkeepers.

Opening of the first hospital pharmacy in Vranje

The first hospital in Vranje was open in a large Turkish-style building called "Suleiman Bey's Sarai" when General Belimarković's troops came to liberate Vranje. The district physician in the hospital was Dr. Franja Kopša, Slovenian, who worked as a military doctor in General Belimarković's army in late 1877 [1]. The district department of Vranje sent two letters to the Ministry of Internal Affairs informing it of the currently poor state of health services in Vranje [3]. The first letter was sent on July 18, 1878, with a request that the Ministry of Internal Affairs appoint a regional physician in Vranje, who would take care of people's health and treat some of the remaining cattle, in accordance with the "*Nastavlenije za okružne lekare i fizikuse*" [1] from 1839, which stipulated that the regional physician was required to perform veterinary services in addition to treating people [4]. Six days later, the District Chief, Mr. Brzaković D. sent another letter to the Ministry of Internal Affairs informing it of the status quo in the only existing pharmacy in Vranje, which was not in accordance with the applicable paragraphs of the Pharmacies and Pharmacists' Act from 1865 [3]. For the only existing felcer Naum's pharmacy, the district physician said that "the pharmacy left in town since the Ottoman period was so messy and unequipped with the basic necessities, that it was barely possible to get the most ordinary items; including those at high prices. The doctor is a man who is unwell prepared for the job, he often does not know how to put together/mix the ingredients given to him in the prescriptions by military doctors ... " [3]. Dr. Franja Kopša was appointed for the district physician, and also the chief of the district hospital on February 2, 1879 [3]. On December 22, 1881 the Garrison Hospital became the first hospital in Vranje District [1]. In the hospital courtyard, in one of the two adjacent restored buildings a small temporary hospital pharmacy was placed so that drugs that Dr. Kopša found in the Military Hospital could be supplied [5]. Medications were used for in-patient and outpatient treatment. The entire population of Vranje was supplied by the drugs from the hospital pharmacy because there were still no city pharmacies with trained pharmacists.

The hospital pharmacy worked until the hospital was moved to the new building and the first modern private pharmacy was opened in 1883 [1.] The number of staff that worked in the military hospital pharmacies was in coordination with the needs of the population, therefore; for instance, among the survived in 1878 was only a pharmacy assistant, a pharmacist Steva Varjačić, who worked there until 1881 [6]. At the time, there were nine medical doctors and physicians in Serbia who were required to respect the rules introduced by the Ordinance for District and Municipal Physicians "*Nastavlenija za okružne lekare i fizikuse*" on August 21, 1839 [7] .

Three unsuccessful attempts to open the pharmacy in Vranje

The autonomy called "Hattisherif" which was acquired in 1830 created favorable conditions for the complete organization of health services in Serbia, and when the Sretenje Constitution was established in 1835, attention paid to the organization of health services started to grow. By the Decree passed on the basis of the Constitution health care for people was provided as well as founding of schools for surgeons and midwives, veterinary doctors and pharmacists [7, 8].

There were also two significant laws passed by Knez Miloš in 1836. It was decided that doctors should treat poor people for free, and in 1837 the sale of medicines was prohibited without the written permission from the police who were to obtain these permissions from a trained doctor [7].

In 1845 it was decided to check all the retail shops in order to forbid selling of any sort of preparations and medicines and all such work was transferred to the pharmacies which were opened at that time. In 1845 the Regulations for Community Pharmacies were adopted (*Pravila za javne apoteke*) as well as for the Managing of the Court Pharmacy (*Pravila o rukovanju i manipulisanju Pravitelstvene apoteke*) [7]. The first real legislation for pharmaceutical activity in relation to the conditions for the establishment of pharmacies was passed by Mihailo Obrenović in 1865 and it was called The Law for Pharmacies and Pharmacists and Keeping Drugs and Poisons (*Zakon za apoteke i apotekare i za držanje i prodavanje lekova i otrova*) [7] . In 1879 The Law Concerning Health Care and Preservation of People's Health (Zakon *o sanitetskoj struci i o čuvanju narodnog zdravlja*) was passed, to be formally accepted only in 1881 [7]. According to this Law, the regional physician used to care and submit a report about the work of the pharmacists in the areas over which they had control. The lack of trained pharmacists in the south of Serbia and especially in Vranje was the reason why Vranje had its first two public pharmacies opened only at the end of the 19th century. A year before the opening of the first pharmacy with a trained pharmacist, Vranje

[1] "Ordinance for District and Municipal Physicians "

had 8,291 inhabitants [9]. The Law Concerning Health Care and Preservation of People's Health (*Zakon o sanitetskoj struci i o čuvanju narodnog zdravlja*) stated that the permission to open and run a pharmacy would be issued at the request of a person concerned in places where a pharmacy was a real necessity, in towns and villages with at least 2,000 inhabitants who didn't have a pharmacy [7].

After the liberation from the Turks (1876–1878) there were additional problems in the organization of health services caused due to the increasing number of population in the liberated districts (Niš, Pirot, Vranje, Toplica). Some pharmacists from Serbia wanted to move their pharmacies to the new liberated districts but they were not allowed [10]. Felcer Naum Marković approached the Ministry of Internal Affairs to legalize his pharmacy in 1879 [11,] while at the same time another request was recorded by an apothecary assistant Nikola Pron who asked the Ministry to grant him a license for the opening of a pharmacy either in Vranje or Leskovac [11]. A foreigner Pron presented himself as a graduate from Austro-Hungarian Monarchy, he also asked the Municipality for support and material help in cash or help by giving him an apartment to live in. The Minister couldn't offer any financial assistance, and after many months of correspondence he finally got his diploma back on January of 1880 [3]. One of the reasons for him being rejected laid in the Pharmacies and Pharmacists Act of 1865 which allowed only pharmacists and chemistry graduates of Serbian origin or foreigners who obtained Serbian citizenship to open and run pharmacies [7]. The other reason was the fact that a lot of community money was already spent for the building of the local high school (finished in summer of 1933) [12]. That is how the first unsuccessful attempt finished leaving Naum's pharmacy as the only one in the municipality of Vranje. According to Mihajlović [13] Naum's pharmacy was soon taken over by a military pharmacist Petar (Pera) Janković who came to Vranje with the Šumadija corps and he worked alone for some time until 1883 occasionally with many pharmacy assistants who worked in the lack of qualified stuff. However, we found an unpublished data from 1879 in the State Archive of Serbia (SAS) which relate to the correspondence between Petar (Pera) Janković and the Ministry of Internal Affairs concerning the application grant for the license (concession) to open his own pharmacy. This correspondence referred to another city – the city of Kragujevac not Vranje. Based on the available preserved data/reports from the SAS, he was granted the concession to start his business in Kragujevac in 1879, but it cannot be concluded with absolute certainty until what month of that year Janković worked in Vranje, *de facto* [11] and when he started working in his pharmacy in Kragujevac. Many SAS data are found in relation to medicine refund requests to the Ministry of Internal Affairs concerning the free treatment/full reimbursement of, convicts and poor high-school students from Kragujevac, the local Community Hospital, in the first five years of his working there [11, 14–17]. Interestingly enough, there is a record from 1879 of his request to be allowed to supply citizens of Vranje and the military hospital in Vranje (later it became a community hospital in Vranje), probably from the period when he still worked in Vranje. His younger brother, Mihailo Mika Janković was also a pharmacist and he opened the second pharmacy in Vranje in 1892 that was at that time working as a pharmacist in Vranje [5]. After Pera Janković's death in 1900 he was succeeded by his brother Mika Janković after he returned the concession for the Pharmacy in Vranje on April 25, 1901 [10]. No record could be found to shed light on the fate of the first pharmacy which people used to call Naum Markovic'pharmacy. This pharmacy is mentioned as Naum Marković's pharmacy in many archive data until 1884, although there are no data that he received a positive answer to his application for getting of the concession for this pharmacy. Namely, he submitted his application in 1879, probably after pharmacist Petar went to Kragujevac, but since he was an uneducated pharmacist he did not meet the requirements for obtaining a concession. Until the opening of Velimir Karić's pharmacy, who was an educated pharmacist in 1883, Naum's pharmacy was the subject of numerous medical authority controls: "Naum Marković's pharmacy in Vranje was inspected and it was found that there are no drugs that are needed" [14]. The head office of Vranje sent an act to the Minister of Internal Affairs informing him that felcer Naum was found for the lack of drugs, and soon the Ministry of Internal Affairs received his appeal against the conviction of Vranje head office [14]. There was a problem in Vranje regarding the opening of the first pharmacy, so that Naum's pharmacy was still the only pharmacy in the town in the year to come. In 1881 the problem of Naum Marković's pharmacy was still there for the Ministry of Internal Affairs [18].

In accordance with the Law on Organization of Medical Profession and Public/National Health Protection from 1881, a title was established by the Inspector of the Health Institute in the Medical Department of the Ministry of Internal Affairs. The first inspector was Mladen Janković, who was asked to supervise the conditions of health institutions in Niš, Knjaževac, Pirot, Vranje, Loznica, and to report all his findings to the Minister of International Affairs [3]. Inspector Mladen Janković visited Vranje in July 1881 and he wrote about his survey of the empirical pharmacy owned by Naum Marković: "There is a pharmacy in Vranje where you can find some medicines but it is poorly equipped. The medicines are not prescribed and it would not be of any use, anyway, because the pharmacist cannot read or write and he has no assistant but a young apprentice. I tried to convince the owner to employ a recently graduated pharmacist as soon as possible and to supply the pharmacy according to the law. If he does that, then the doctor will prescribe medicines from his pharmacy, but if he doesn't accept my suggestions then the pharmacy will have to be closed" [3].

In the magazine "National Health" number 40 of March 1881 in the article titled "The Medical Personnel" the answer to the question "Are there pharmacies and how are they arranged? " was that "the number of pharmacies increased for four in 1880. While in 1879 there were 28 public pharmacies, at the end of 1880 there were 32. Physicians say that all are good, while for the one in Vranje they say "fairly good".

It goes without saying that private and hand pharmacies of county doctors are not taken into account. All the apothecaries are mostly graduated pharmacists, only for the one in Vranje the regional physician said: "an absolutely ignorant person who doesn't even know how to sign his name, or how to measure and without any knowledge of Latin language... (...) therefore, this pharmacy was closed and a new tender was announced" [19].

The second unsuccessful attempt to open a pharmacy in Vranje was a request of a pharmacist Jovan Šteker submitted on October 6, 1880 for obtaining a permit to open a pharmacy in Vranje [18] (Figure 1). According to the Health Law of that time, valid for the pharmacies and pharmacists, possession and selling of drugs and poisons, if a pharmacist did not open a pharmacy within a year he would lose the right to open it. Seeing that a year expired and that he still hadn't put his pharmacy in order, a pharmacist Jovan Šteker once again addressed to the Ministry of Internal Affairs with a letter requesting the deadline to be extended for a month on September, 11 1881 [3]. His request was rejected, which meant he lost the right to open a pharmacy. That was how the only pharmacy of felcer Naum continued to operate [3].

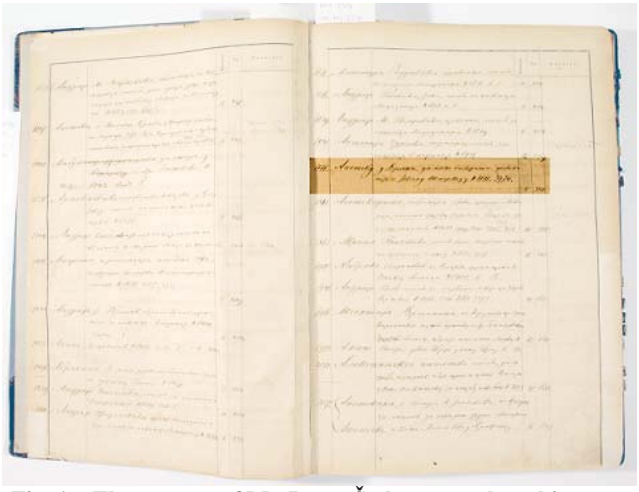


Fig. 1 – The request of Mr Jovan Šteker to prolong his concession for opening of the first pharmacy in Vranje in 1881 – the source originated from the State Archive of Serbia
(The Ministry of Internal Affairs, Sanitary Department. SN 1771, Belgrade, 1881).

Establishment of the first community pharmacy and its owners

The development of education in pharmacy practice in Vranje started with coming of an educated pharmacist Velimir Karić, who in 1883 got a concession (state approval for the management of a pharmacy which referred to a given pharmacist and true for the municipality, city or location) and opened the first pharmacy in Vranje [3].

Such a terrible situation in the pharmacy branch in Vranje region needed to be stopped, and pharmaceutical activity harmonized with the (former) Law of that time. Presumably, on the request of Dr. Mladen Janković, the inspector of the Sanitary Department of the Ministry of Internal Affairs "an announcement for opening of a pharmacy in Vranje" was published on October 26, 1881, which read: "Under Article 24, point 2, second indent of the Act of March 30 of this year, dealing with the arrangement of medical profession and preservation of public health, The Minister of the Internal Affairs publishes this announcement for the opening of a pharmacy in Vranje. We invite all those who have the ability to hold/manage a pharmacy specified in paragraph 6 of the Article 24 ("to open and maintain a pharmacy shall be allowed only to those pharmacists who present valid master's degree in pharmacy and at the same time consistently proved to have been overzealous in pharmacy practice and good governance, to enjoy Serbian citizenship, and to have wherewithal to open a regular pharmacy") to report to the Ministry of the Internal Affairs with all the documents which are required by paragraph 6 of the Article 24 of the said Act not later than November, 15 of this year. The priority to the concession belongs to the sons of this country, then to the Serbs from abroad, and only if those were unavailable, then other options would be considered [19] (Figure 2).

Velimir Karić applied to the announcement [15]. He was a pharmacist born in a clerk's family in Kragujevac in 1859 [1]. On the list submitted by the Ministry of Internal Affairs to the Ministry of Defence, which asked for a report on the number of pharmacies and the number of staff, because of an urgent scheduling of military-medical personnel during April 1876, there was Velimir Karić as a seventeen-year-old prac-

О Г Л А С И.

Стечај.

за отварање апотеке у Врању.

На основу члана 24-ог тачке 2-ге, алинеје друге закона од 30-ог Марта о. г. о уређењу санитетске струке и о чувању народног здравља, Министар унутрешњих дела расписује за отварање апотеке у Врању овај стечај.

Позивају се сви они, који имају способности за држање апотеке, прописане тачком 6-том члана 24 ог («отворити и држати апотеку дозволиће се само оним апотекарима, који поднесу пуноважну диплому магистра фармације, и који у исто време осведоче да су били приљежни у практици апотекарској и доброг владања, да уживају српско грађанско право, да имају потребан новац за отварање уредне апотеке») да се најдаље до 15 ог Новембра ове године пријаве Министарству унутрашњих дела са свима документима, који се траже тачком 6-том члана 24-ог поменутог закона.

Првенство на концесију имају претходно синови ове земље, за тим Срби са стране, и кад ових не би било, онда остали по избору.

Сѫ 4090 Из санитетског одељења Министарства унутрашњих дела.

ШТАМПАНО У ДРЖАВНОЈ ШТАМПАРИЈИ У БЕОГРАДУ.

Fig. 2 – Official paper announcement for the opening of a pharmacy in Vranje
[Source: The State Archive of Serbia – Narodno zdravlje 1881; 21 (October 26, 1881)].

titioner in Mase pharmacy of late Mr Filipović in Šabac, where, "he has practiced for 2 years and 8 months; he did not finish school but he can be used as a pharmacy assistant". Velimir Karić was entered as a pharmacy assistant onto the list of 42 other pharmacists and pharmacy staff who took part in the first Serbian-Turkish war in 1876–1877 [6].

He studied pharmacy in Vienna, and after graduating in 1882, he got a concession to open the first pharmacy in Vranje. After acquiring the concession, within the statutory time of a year he started to prepare and organize his pharmacy. While preparing the opening of his pharmacy, Karić experienced minor unpleasantness from felcer Naum. When in the early autumn of 1882 he completed the arrangement of his pharmacy, he sent a letter to the Ministry of Internal Affairs on October 6, asking: "Please close the pharmacy of Mr Naum Marković since I Velimir Karić am ready to open the pharmacy for which I got the concession" [15]. It meant that his pharmacy, which he acquired by concession, was ready for the opening. On October 21, 1882, the Ministry of Internal Affairs corresponded to the district officers in Vranje: "Recommend Naum to settle with Karić, or all his drugs would be confiscated" [13]. In accordance with the former practice during the liquidation of the pharmacy, the new pharmacist came to an agreement with the doctors who owned the hand pharmacies about an agreeable repurchase of all the existing drugs. Felcer Naum and the pharmacist Karić carried out the liquidation of the existing pharmacy. After that, on December 17, in 1882 the pharmacist Karić officially asked for a commission of the Ministry of Internal Affairs which was to accomplish control of the newly opened pharmacy. In the bookkeeping journal of the Sanitary Department of the Ministry of Internal Affairs under the number 6708 of December 17th, 1882 it says "It was approved through the district office to keep the pharmacy and the commission/board will be appointed" [15]. Velimir Karić's pharmacy began to work on January 27, 1883; 27 days after the Ministry gave its permission [2]. The commission which was supposed to inspect the pharmacy and give an official permission for its work started from Belgrade only towards the end of 1883, headed by a state chemist Dr Šams, and in each place in Serbia they formed commissions after the instructions given by the Ministry of Internal Affairs. As for Vranje, there is no available information saved on *de jure* release of the pharmacy. It was recorded that after the inspection of the pharmacies in Palanka, Ćuprija, Aleksinac, Niš, Leskovac and Vranje in 1884, only the subject-inspection of Velimir Karić's pharmacy was transferred under the number N 9217 for 1894 [17].

It can be considered that the inspection of Vranje pharmacy was completed by the end of January 1884, when the pharmacy was also put into operation *de jure* [3]. During the research in the bookkeeping journal of the Ministry of Internal Affairs for 1883 we found several references to Karić pharmacy such as an invoice for the medicines bills. One invoice was for the dispensed medicines for January, February, March and April to the County Hospital in Vranje, and the other one for the following months issued on August, 18 1883 [16]. As a very prominent figure in Vranje and Vranje Region he was elected a Member of Parliament in 1897. He was a member of the parliamentary delegation which brought King Petar Karađorđević from Geneva in 1903. Due to his political activities he decided to move to Belgrade, and in 1905 he returned the concession for Vranje and took over a pharmacy in Belgrade. After going to Belgrade, Velimir Karić became the president of the Serbian Apothecary Society, where in 1919 participated in a decision making to purchase all the necessary medications abroad as soon as possible and to distribute the same to the pharmacies in the country [20].

City pharmacy's owners

The first pharmacy in Vranje changed owners frequently in the first three decades of its operation. Pharmacist Jovan Jovanović took over the first pharmacy in Vranje from Velimir Karić after his departure to Belgrade. Pharmacist Jovanović completed his pharmacy studies in Gratz in 1890. He worked in Vranje from 1906 to 1910. He was noticed for consulting that he gave to chronic patients, and for his humane attitude towards the poor. He died in 1912 [13].

Pharmacist Borivoje Marić became the third owner of the first pharmacy in Vranje, who took over the pharmacy from Jovan Jovanović in 1911 [13]. He was born in Smederevo and completed his pharmacy studies in Gratz. He came to work in Vranje just before the beginning of the Balkan War, when there was the devastating epidemic of typhoid in Vranje and the whole town was turned into a refugee camp. He gave the poor patients and the refugees medicines for free. Mr. Marić's pharmacy did not work during the Bulgarian occupation. After the liberation in 1922, pharmacist Marić handed his pharmacy over to his college Aleksander Devedžić, the first pharmacy owner from Vranje [12], while he went to live and work in Paraćin [11].

Aleksandar Devedžić was the first man from Vranje who started the pharmacy business in the first pharmacy in Vranje (Figure 3). Although there are no archive proofs about its location, it is supposed that Mr. Velimir Karić's pharmacy was on the same place where the pharmacy of Mr. Aleksandar Devedžić.

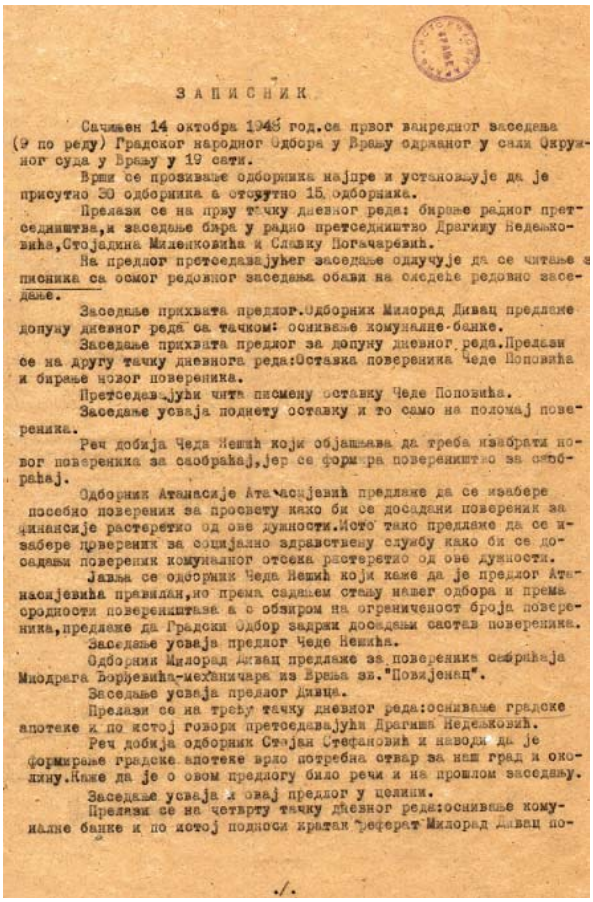
He was born in Vranje in 1895 and graduated in Zagreb in 1922 [13]. After graduating he immediately took over the first pharmacy in Vranje from his colleague Bora Marić. Canko's pharmacy, as it was called by the people in Vranje, was remembered as a benefactor's and healer's pharmacy, where you could heal all human pain. After eight years of work Aleksandar moved to Skopje and he handed his pharmacy to be ran by Stojadin Milenković [12]. However, after the liberation in 1945, Devedžić returned to Vranjska Banja where he was appointed the manager of Vranjska Banja. He began to work on the opening of the first pharmacy in Vranjska Banja, but his sudden death in 1964 prevented him from seeing its realization. Aleksandar Devedžić was the manager of the pharmacy in Bosilegrad till the end of October 1960 [21].

Pharmacist Stojadin Milenković was born in Vranje, he finished pharmacy studies in Zagreb in 1924. Like the previous pharmacists, first of all he opened a cosmetic laboratory where he was making a large number of different cosmetic

Fig. 3 – The pharmacy of Aleksandar Devedžić – the fourth owner of the first pharmacy in Vranje who worked and owned the pharmacy between 1922–1930 – supposed to be the pharmacy on the same location as it was later on the pharmacy of Mr Velimir Karić.

(Source: Historical Archive of Vranje "January 31" Vranje: Administration and Public Services, Municipal Assembly).

products. Stojadin Milenković was elected a member of the City National Board in Vranje on October 14, 1948, and he took part in the adoption of the proposal about the opening of the People's Pharmacy in Vranje (Figure 4) [22]. His pharmacy had the newest medicines. In 1949 he gave his pharmacy to social control, and he took over the management of one of the pharmacies that were in the social management. He worked in the People's Pharmacy in Vranje from July 13, 1949 to October 15, 1963 when he moved to Belgrade with his family [23].



З А П И С Н И К

Сачињен 14 октобра 1948 год.са првог ванредног заседања (9 по реду) Градског народног Одбора у Врању одржаног у сали Окружног суда у Врању у 19 сати.

Врши се прозивање одборника најпре и установљује да је присутно 30 одборника а отсутно 15 одборника.

Прелази се на прву тачку дневног реда: бирање радног председништва,и заседање бира у радно председништво Драгишу Недељковића,Стојадина Миленковића и Славку Погачаревић.

На предлог претседавајућег заседање одлучује да се читање записника са осмог редовног заседања одави на следеће редовно заседање.

Заседање прихвата предлог.Одборник Милорад Дивац предлаже допуну дневног реда са тачком: оснивање комуналне-банке.

Заседање прихвата предлог за допуну дневног реда.Прелази се на другу тачку дневнога реда:Останка повереника Чеде Поповића и бирање новог повереника.

Претседавајући чита писмену оставку Чеде Поповића.

Заседање усваја поднету оставку и то само на поломај повереника.

Реч добија Чеда Нешић који објашњава да треба изабрати новог повереника за саобраћај,јер се формира повереништво за саобраћај.

Одборник Атанасије Атанасијевић предлаже да се изабере посебно повереник за просвету како би се досадани повереник за финансије растеретио од ове дужности.Исто тако предлаже да се изабере повереник за социјално здравствену службу како би се досадањи повереник комуналног отсека растеретио од ове дужности.

Јавља се одборник Чеда Нешић који каже да је предлог Атанасијевића правилан,но према садашњем стању најмег одбора и према сродности повереништава а с обзиром на ограниченост броја повереника,предлаже да Градски одбор задржи досадањи састав повереника.

Заседање усваја предлог Чеде Нешића.

Одборник Милорад Дивац предлаже за повереника саобраћаја Миодрага Ђорђевића-механичара из Врања зв."Повређен".

Заседање усваја предлог Дивца.

Прелази се на трећу тачку дневног реда:оснивање градске апотеке и по истој говори претседавајући Драгиша Недељковић.

Реч добија одборник Стојан Стефановић и наводи да је формирање градске апотеке врло потребна ствар за наш град и околину.Каже да је о овом предлогу било речи и на прошлом заседању.

Заседање усваја и овај предлог у целини.

Прелази се на четврту тачку дневног реда:оснивање комуналне банке и по истој подноси кратак реферат Милорад Дивац по-

./.

Fig. 4 – Establishment of the state-owned city pharmacy in Vranje on October 14, 1948. (Source: Historical Archive of Vranje "January 31" Vranje. Administration and Public Services, Municipal Assembly).

Conclusion

Most of the resources we consulted proved that the pharmacy of Mr. Velimir Karić was the first one with the educated pharmacist opened according to the decision of the Ministry of Internal Affairs in 1883. Yet, it may be argued with a great historical certainty that even before that period there had been a pharmacy in Vranje, held by unsatisfactory educated authority, felcer Naum Marković. Over a very short period of time, between 1878–1879, there worked the educated pharmacist, Petar Janković, who came to Vranje with military corps. The matter of expertise in pharmaceutical preparation in such an apothecary shop was on the agenda many times when Naum Marković's pharmacy was inspected. Upon all the results and foundings it could be concluded that opening the pharmacy of Karić was a turning point in the development of pharmacy in the district. Although there are no archive records where Mr. Velimir Karić located his pharmacy shop in the city, it has to be supposed that the pharmacy of Mr. Velimir Karić was on the same place as the pharmacy of Mr. Aleksandar Devedžić. Hence, we found no records of the change of the apothecary shop location in that time. It was privately owned until the Nationalization Law in 1949. Besides, Mr. Karić, the pharmacists working with the Military Hospital (later transformed into the County Hospital with a separate military pharmacy operated from 1878 to 1883) were also preparing and supplying the medicines for the outpatients, so it could be concluded that the citizens of Vranje did not lack safe treatment and treatment outcomes in this region.

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E R R A T U M

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Results should be presented in logical sequence in the text, tables and illustrations. Emphasize or summarize only important observations.

Discussion is to emphasize the new and significant aspects of the study and the conclusions that result from them. Relate the observations to other relevant studies. Link the conclusions with the goals of the study, but avoid unqualified statements and conclusions not completely supported by your data.

References

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

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

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

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Primeri referenci:

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

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

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

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